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HYGIENIC LABORATORY.—BULLETIN No. 42.
January, 1908.

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HERMAL DEATH POINTS OF PATHOGENIC MICRO-ORGANISMS IN MILK

By

M. J. ROSENAU,
Director Hygienic Laboratory.

WASHINGTON:
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Biological
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CONTENTS.

Page.
Introduction.................................... 7
Methods.................................... 9
Bacillus tuberculosis.................................... 10
  Original experiments.................................... 10
  Conclusion.................................... 30
  The effects of dead tubercle bacilli.................................... 30
  Review of the work of others upon the thermal death point of the tubercle bacillus.................................... 34
Bacillus typhosus.................................... 48
  Review of the work of others.................................... 48
  Original experiments.................................... 51
  Conclusion.................................... 56
Bacillus diphtheriæ.................................... 56
  Review of the work of others.................................... 56
  Original experiments.................................... 57
  Conclusion.................................... 61
Vibrio choleræ.................................... 62
  Review of the work of others.................................... 62
  Original experiments.................................... 63
  Conclusion.................................... 71
Bacillus dysenteriæ.................................... 71
  Review of the work of others.................................... 71
  Original experiments.................................... 71
  Conclusion.................................... 78
Micrococcus melitensis.................................... 79
  Review of the work of others.................................... 79
  Original experiments.................................... 79
  Conclusion.................................... 80
General summary and conclusions.................................... 81

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THE THERMAL DEATH POINTS OF PATHOGENIC MICRO-ORGANISMS IN MILK.[a]

By Milton J. Rosenau,

Surgeon and Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

The temperature at which milk should be pasteurized hinges on the thermal death points of the pathogenic micro-organisms which contaminate it. The pathogenic micro-organisms most frequently found in market milk are those causing tuberculosis, typhoid fever, diphtheria, scarlet fever, dysentery, and Malta fever. Fortunately none of the organisms causing the above-mentioned diseases has resisting spores. Moderate degrees of heat are, therefore, sufficient to render milk safe so far as these dangers are concerned. The streptococci, staphylococci, and most of the bacteria associated with infantile diarrhea are also readily destroyed by heat.

Although it would appear to be a comparatively simple matter to determine precisely the temperature at which micro-organisms die, such work is in fact surrounded by many difficulties and pitfalls; different investigators have come to widely different results. Some of these discrepancies are only apparent and may be explained by the relation of time to temperature. The longer the time of exposure, the lower the temperature necessary to kill any organism. Differences in methods are also responsible for differences in results. Thus some workers start with the fluid containing the micro-organisms to be tested at room temperature and raise it to the desired temperature; others introduce the bacteria after the fluid has been heated.

Care must be taken in thermal death-point work to see that the entire volume of the water bath, as well as the fluid medium tested, has approximately the same temperature throughout. Unless attention to this point is given, a difference of 5° to 10° C. between the top

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[a] Manuscript submitted for publication February 11, 1908.

and bottom layers of the fluid may be found and misleading results obtained. Again, unless a reasonably accurate thermometer is used further inaccuracies may result, for ordinary thermometers not infrequently have errors of several degrees.

. Among bacteria some strains or races are more resistant to heat than others. These differences, which correspond to similar known variations in all animal and vegetable species, must be taken into account.

The individual micro-organisms in any particular culture vary so far as their resistance to heat is concerned. Not all the bacteria are killed at once. Thus it will be seen from the tables that the vast bulk of the bacteria succumb at a certain temperature, while a few hardy individuals resist several degrees higher heat or several minutes longer exposure. It is evident that when we reach the point where only a few organisms remain alive in the fluid, the results may be irregular or vary, depending upon the amount of fluid transplanted into the culture medium.

The physical and chemical composition of the fluid in which the tests are made also exerts a decided influence. Some bacteria are sensitive to acid, others to alkali. If the tests are made in pure water, we have to deal with differences in osmotic pressure (plasmolysis). If the tests are made in an albuminous, viscid, or fatty medium, protecting envelopes may form and act as insulating coverings.

Evaporation takes place so rapidly from exposed fluids that the surface layer may remain cooler than the body of the liquid. This is especially a matter of concern with milk, which sometimes forms a scum above 60° C., owing to rapid evaporation of the surface layer. This scum consists of coagulated albumins in which is enmeshed much fat. The bacteria entangled in this surface pellicle may escape the heat indicated by the thermometer in the deeper layers.

A matter of very great importance is the suitability of the medium into which the bacteria are planted, as well as the temperature and the other conditions of incubation, etc. The optimum conditions may only be realized by a special study of each micro-organism tested. Controls must always show prompt and vigorous growth.

The concentration of the suspension and other differences in the conditions of the experiment account for some of the variations in the results recorded by different observers. If a test tube is used, care must be taken that the glass be thin and that the test fluid be well below the surface of the heated water. It is especially important to prevent soiling of the walls of the test tube above the fluid. This may give false results, for the reason that the pipette or instrument used to withdraw the milk or whatever fluid is used may come into contact with the upper portion of the test tube and thereby pick up

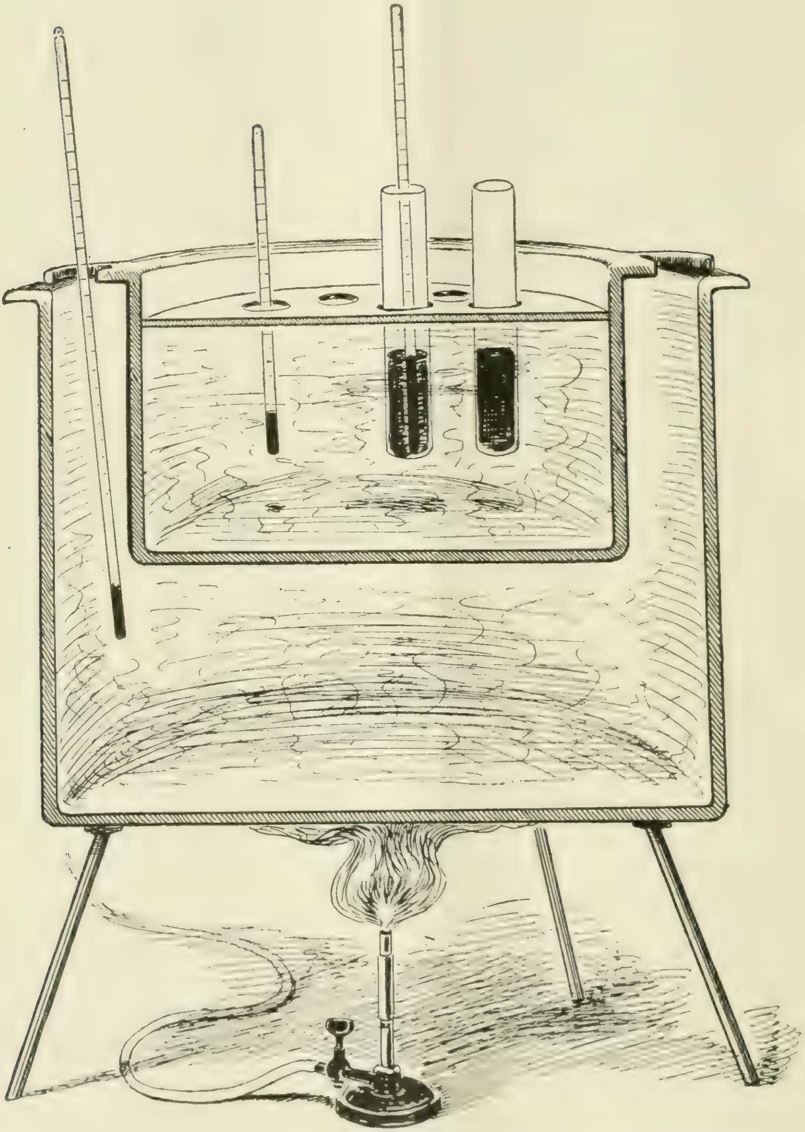

FIG. 1.—APPARATUS USED TO DETERMINE THE THERMAL DEATH POINTS OF PATHOGENIC BACTERIA IN MILK.

bacteria which may have escaped the heat, or the fluid upon the side of the test tube may afterwards flow back.

As a rule, bacteria are attenuated and lose their power to infect before they lose their ability to vegetate upon artifical culture media. It is, therefore, safe to assume that a micro-organism that will not grow in artificial media under favorable conditions is "dead." The tubercle bacillus is an exception to this rule, for reasons given further on.

METHODS.

The methods used in the tests recorded below were planned to imitate the actual conditions of pasteurization, so far as practicable, in laboratory experiments.

Except in the case of the tubercle bacillus, all the organisms were first planted in sterilized milk and not subjected to the tests until an abundant growth was obtained. In order to obtain pure cultures it was first necessary to sterilize the milk, which was done by the fractional method. It is believed that this does not appreciably influence the results obtained. By growing the micro-organisms in milk we perhaps imitate the natural conditions more closely than by using suspensions prepared for the purpose.

All the tests were made by placing the test tube containing the cool infected milk into the water bath at the desired temperature, usually 60° C. The time required for the milk to reach the temperature stated is always given.

The illustration shows how the apparatus was arranged for all the tests. The temperature was controlled within a fraction of a degree; it never varied more than half a degree, and, as a rule, was kept within one or two tenths of a degree. The test tubes containing about 10 to 15 c. c. of milk were immersed so that the level of the milk in the tube was well below the level of the water.

The reaction of the milk was determined by titration with $\frac{N}{10}$ NaOH solution, using phenolphthalein as indicator, and the results are expressed in terms of lactic acid. The reaction of the milk was always taken at the time of testing the thermal death points.[a]

Enrichment methods were used only in the case of cholera, but did not materially alter the results of the simpler methods.

The tests were made with both whole and skimmed milk. As a rule, the milk was first partly skimmed in accordance with the usual bacteriologic technic in preparing this culture fluid. Whether whole milk or skimmed milk was used is stated in each instance.

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[a] I am indebted to Dr. Joseph H. Kastle, Chief of the Division of Chemistry, for making these tests, as well as for determining the chemical composition of the milks used in the work on *B. tuberculosis*. I am also indebted to my colleagues in the Division of Pathology and Bacteriology, especially Doctors McCoy, Stimson, Miller, King and Anderson, for assistance with the tests thorughout this work. Further I desire to express my special obligations to Doctor Stimson for assistance in collecting the literature upon the thermal death point of the turbercle bacillus.

The test tubes in which the infected milk was heated were open to the air, and scum formation was disregarded in all instances, my object being to determine the thermal death point against natural difficulties, so that the results might be applied with confidence to practical pasteurization.

BACILLUS TUBERCULOSIS.

Certain special difficulties are met with in determining the thermal death point of the tubercle bacillus. This organism does not grow readily upon artificial media. The few experiments made to determine its thermal death point by cultural methods have no significance, because its vegetability upon artificial media does not correspond to its power of growing in the animal organism. It is therefore necessary to inoculate animals in order to determine whether or not the tubercle bacillus is alive and virulent. Here again we meet with complications. Dead tubercle bacilli have a certain amount of pathogenic power and produce lesions, including tubercle formation, abcesses, and coagulation necrosis. However, while we lack a criterion to determine with precision the exact point when the tubercle bacillus dies, we are able by means of animal inoculations to determine just when the tubercle bacillus is so enfeebled that it is no longer able to infect. This, after all, is the important practical point.

ORIGINAL EXPERIMENTS.

In all the tubercle experiments raw whole milk was used, the chemical composition of which is stated in each case. The number of bacteria other than the tubercle bacillus was determined by the usual method on agar plates.

The young tubercle cultures were rubbed up with the milk in a mortar until a fairly uniform suspension was obtained. Otherwise the methods used were those given on page 9.

Nine experiments were made with five different bovine cultures, the details of which will be found in the following tables. The results are consistent so far as the thermal death point is concerned. The only irregularity will be found in Table No. 1, guinea pig No. A12. This animal did not have lesions differing markedly from those caused by dead tubercle bacilli; but the secondary guinea pig ($A12^1$) died as a result of generalized tuberculosis, affecting especially the lungs. The secondary guinea pig, inoculated with the lesions of A9, remained well. The other animals of this series, namely, A7, A8, A10, and A11, evidently did not have an active tuberculosis due to living and virulent tubercle bacilli.

TABLE No. 1.—*Series A.*

Bovine culture, H, from Leonard Pearson.

This culture was isolated July 5, 1899, from the omentum of a guinea pig that had been inoculated with an emulsion of caseous material from the mesenteric gland of a Jersey cow 7 years old. The culture was grown on blood serum for 22 generations, then transplanted on glycerin-agar. The culture used was either the thirty-third or thirty-fourth generation on glycerin-agar. The culture was virulent for guinea pigs, rabbits, and cattle.

Twenty cubic centimeters of a mixed market milk was heavily inoculated from two subcultures on potato.

Under the microscope many tubercle bacilli lying singly and in clusters were found in each field.

Composition of the milk: Specific gravity, 1.031; fat, 5.2; total solids, 13.62; solids not fat, 8.42; ash, 0.73; acidity, 0.149; refractometer, 41; sediment (by volume), 0.07; milk sugar, 4.18; no coloring matter; no preservatives.

This milk contained 840,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. of the infected milk injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
A1	Control; 28° C. (at start).	260	330	Died on 21st day	Generalized tuberculosis.	Inguinal glands on both sides enlarged and caseous; general miliary tuberculosis of peritoneum; liver enlarged and has few whitish nodules; spleen about twice normal size, no distinct tubercles visible; few tubercles in upper lobe left lung; anterior mediastinal glands swollen, not caseous.
A2	....do	250	220	Died on 23d day	....do	Emaciation; inguinal glands enlarged and caseated; general miliary tuberculosis of peritoneum; liver much enlarged, contains few whitish nodules; spleen four times normal size, with few whitish areas; omentum rolled up into large caseous mass; few small tubercles in lungs.
A3	60° (took 4 minutes to heat from 28° to 60°).	260	250	Died on 52d day	....do	Emaciation; both inguinal and axillary glands much enlarged; nodule size of pea in mesentery; spleen four times normal size and tuberculous; liver and lungs contain advanced tubercles.
A4	1 minute at 60°	260	325	Chloroformed 55th day	....do	One inguinal gland enlarged; caseous abscess in abdominal wall at site; several nodules scattered on peritoneum; omentum has numerous tubercles;

						liver and spleen both have caseous nodules; anterior mediastinal glands enlarged and caseous; lung contains one small nodule.
A5	5 minutes at 60°	265	375	....do	Tuberculosis (?)	Small noncaseous nodule in abdominal wall at site; few nodules not caseous in omentum. *A secondary guinea pig* inoculated with emulsion of lesions and glands (site, omentum, spleen, liver, and lung of A5) developed generalized tuberculosis and died in 45 days.
A6	....do	270	436	Died in 8 hours following injection of tuberculin (2 c. c.) on 97th day.	Generalized tuberculosis.	Omentum thick as one's little finger and caseous; celiac group of glands large and caseous; anterior mediastinal glands enlarged; yellow nodules in substance of liver, spleen, and kidney.
A7	10 minutes at 60°	275	360	Chloroformed 55th day	Negative	Inguinal glands enlarged; small nodule, not caseous, in abdominal wall at site; few noncaseous nodules in omentum.
A8	....do	275	575	Chloroformed 98th day	....do	Seemed slightly sick following subcutaneous inoculation 2 c. c. tuberculin, but recovered; normal throughout at autopsy.
A9	15 minutes at 60°	280	370	Chloroformed 55th day	....do	Small caseous abscess at site in abdominal wall; small nodule in mesentery; several smaller nodules on peritoneum posteriorly; spleen normal but bound to surrounding parts; omentum contains numerous nodules, some caseous. *A secondary guinea pig* inoculated into the peritoneal cavity with an emulsion of the lesions and apparently normal glands of A9 (viz, site, liver, spleen, peritoneal nodules, and omentum) remained normal and gained weight; when tested with 2 c. c. tuberculin on the 108th day it did not respond; no lesions at autopsy.
A10	....do	280	600	Chloroformed 98th day	....do	Seemed sick, but did not die, as result of subcutaneous inoculation 2 c. c. tuberculin; few hard nodes in omentum.
A11	20 minutes at 60°	280	300	Chloroformed 55th day	....do	Caseous abscess in abdominal wall at site; noncaseous nodules in omentum; spleen bound to surrounding parts, otherwise normal; nodules on diaphragmatic peritoneum; otherwise normal.
A12	....do	280	300	Died in 9 hours following injection of tuberculin (2 c. c.) on 97th day.	Negative (?)	Caseous nodes in omentum; celiac group of glands size of a marble; anterior mediastinal glands enlarged; yellowish nodules in liver and spleen. *A secondary guinea pig* inoculated into the peritoneal cavity with the lesions and glands of A12 developed generalized tuberculosis, especially of the lungs; died in 54 days.

Table No. 2.—*Series B.*

Bovine culture, IXb2, from Theobald Smith.

Twenty cubic centimeters of market milk heavily inoculated from a glycerin-agar slant.

Stained smears showed numerous bacilli in each field, also a few small clumps.

Composition of the milk: Specific gravity, 1.0308; fat, 3.7; total solids, 12.3; solids not fat, 8.6, ash, 0.63; acidity, 0.15; refractometer, 40.3; sediment, 0.07; milk sugar, 4.81; no coloring matter; no preservatives.

This milk contained 2,440,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and 1,380 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
B1	Control; 25° C. (at start).	255	235	Died on 23d day....	Generalized tuberculosis.	Emaciation; tuberculosis of inguinal glands, peritoneum, liver, spleen, lungs, mediastinal glands, and omentum.
B2	60° (took 4½ minutes to heat from 25° to 60°)...	255	425	Died in 7 hours following injection of tuberculin (2 c. c.) on 82d day.	...do..........	Inguinal glands enlarged; mesenteric glands size of small marble near pylorus, not caseous; tuberculosis of spleen; anterior mediastinal gland enlarged; ileolumbar gland enlarged and caseous.
B3	1 minute at 60°.......	255	425	Died in 4 hours following injection of tuberculin (2 c. c.) on 82d day.	...do..........	Emaciation; inguinal, axillary and mediastinal glands caseous; large caseous nodule at site; spleen and liver full of tubercles; nodules on peritoneum and in lungs.
B4	7 minutes at 60°......	260	250	...do..................	...do.............	Inguinal glands large and caseous; nodules on peritoneum; spleen and liver full of tubercles; retroperitoneal glands caseous; omentum full of hard nodules; anterior mediastinal glands enlarged.
B5	10 minutes at 60°......	260	315	....do..............	.....do...............	Inguinal and mediastinal glands enlarged; nodules on peritoneum; retroperitoneal glands caseous; tubercles in liver, spleen, testicles, and lungs.
B6	12 minutes at 60°.....	265	425	Died in 4 hours following injection of tuberculin (2 c. c.) on 93d day.	.....do..........	Inguinal, axillary, and mediastinal glands enlarged; tubercles on peritoneum, and in spleen, liver, omentum, and lungs.
B7	15 minutes at 60°........	265	500	Chloroformed 82d day....	Negative.............	No evidence of tuberculosis at autopsy.

B8	18 minutes at 60°	265	475	Died in 12 hours following injection of tuberculin (2 c. c.) on 82d day.	....do	Do.
B9	20 minutes at 60°	270	415	Died in 15 hours following injection of tuberculin (2 c. c.) on 82d day.	....do	A small caseous focus in one ileolumbar gland; otherwise normal.

TABLE No. 3.—*Series C.*

Bovine culture, IXb2, from Theobald Smith.

Twenty cubic centimeters of market milk heavily inoculated from a glycerin-agar slant.

Stained smears showed numerous bacilli in each field, also occasional clumps.

Composition of the milk: Specific gravity, 1.0308; fat, 3.7; total solids, 12.3; solids not fat, 6; ash, 0.63; acidity, 0.15; refractometer, 40.3; sediment, 0.07; milk sugar, 4.81; no coloring matter; no preservatives.

This milk contained 2,440,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and 1,380 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
C1	Control, 29° C. at start.	300	255	Died on 19th day.	Generalized tuberculosis	Emaciation; cheesy nodule at site; tuberculosis of inguinal and axillary glands, peritoneum, omentum, lungs, and pleura.
C2	do.	270	215	do.	do.	Emaciation; tuberculosis of inguinal, axillary, mesenteric and mediastinal glands; liver and spleen enlarged and full of nodules; omentum rolled up into one large caseating mass; tubercles in both lungs.
C3	60° (took 11 minutes to heat from 29° to 60°).	290	250	Died on 24th day.	do.	Emaciation; caseous nodule at site; inguinal glands enlarged; general miliary tuberculosis of peritoneum; whitish and yellow nodule in spleen and liver; abdominal and mediastinal glands caseous; omentum rolled up into a hard mass with softened areas.
C4	65° (took 5 minutes to heat from 29° to 65°).	285	270	Died on 29th day.	Negative.	No evidence of tuberculosis at autopsy; inguinal glands moderately enlarged; adhesions between omentum and abdominal wall, stomach dilated; otherwise normal.
C5	1 minute at 65°.	300	600	Chloroformed 93d day.	do.	Did not react to 2 c. c. tuberculin; pregnant; caseous nodule in omentum; otherwise normal.
						Secondary guinea pig inoculated intraperitoneally with emulsion of omental nodules, pancreas, etc., of C5 gained weight, did not respond to 2 c. c. tuberculin on 69th day; autopsy negative.

C6	5 minutes at 65°	300	550	....do	....do	Became sick, but did not die following tuberculin; normal at autopsy excepting a nodule, not caseous, in pancreas. *Secondary guinea pig* (same details as C5), negative.
C7	8 minutes at 65°	325	600	Died on 90th day	....do	Streptococcal double pneumonia; no evidence of tuberculosis at autopsy.
C8	10 minutes at 65°	325	520	Chloroformed 93d day	....do	Did not react to tuberculin; normal at autopsy except a caseous enlargement in omental pancreas, with adhesions to abdominal wall. *Secondary guinea pig* (same detail as C5), negative.
C9	15 minutes at 65°	330	520	Died in 9 hours, following injection of tuberculin (2 c. c.) on 93d day.	....do	Well-preserved, pregnant; excess peritoneal fluid; small caseous abscess at site; few nodules in omentum, some softened; adhesions between liver and stomach. *Secondary guinea pig* (same details as C5), negative.

TABLE NO. 4.—*Series D.*

Bovine culture, Bovine III, from Department of Agriculture.

Twenty cubic centimeters special market milk rubbed up with culture.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, few in clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk contained 2,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

The temperature during this experiment averaged from 59.7° to 60.3° C.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
D1	Control; 24.5° C. (at start).	225	190	Died on 11th day	Generalized tuberculosis.	Emaciation; inguinal glands enlarged; 1 anterior mediastinal gland caseous; nodules on peritoneum; omentum many nodules, some size of pea, none softened; spleen fibrinous peripheric splenitis.
D2	do	230	190	Died on 27th day	do	Emaciation; excess fluid in peritoneal cavity; peritoneum studded with tubercles; caseous mass at site; omentum rolled up, large, hard, and caseous; liver, intestines, spleen, etc., bound together; lungs contained tubercles; inguinal, axillary, and mesenteric glands enlarged.
D3	60° (took 5½ minutes to heat from 24.5° to 60°).	235	335	Died in 6 hours, following injection of tuberculin (2 c. c.) on 61st day.	do	Hard nodules at site, not caseous; inguinal glands enlarged; excess clear fluid in peritoneal cavity; small nodules on peritoneal surface; omentum contains soft nodules; liver, spleen enlarged, and full of tubercles; anterior mediastinal gland enlarged; tubercle in lung.
D4	2 minutes at 60°	235	400	Died in 6 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Inguinal and axillary glands enlarged, caseous; excess clear fluid in peritoneal cavity; spleen, liver enlarged, full of tubercles; omentum, several hard caseous nodules; caseous tubercles on testicle; mediastinal and bronchial glands large, caseous; ileolumbar gland involved; tubercle in lung.
D5	5 minutes at 60°	240	365	Died in 5 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Inguinal and axillary glands enlarged; diaphragm studded with nodules; liver mottled with large yellow areas; spleen large and numerous tubercles; caseous nodules in omentum; pancreas, mediastinal, bronchial, and mesenteric glands much enlarged.
D6	10 minutes at 60°	245	450	Died in 14 hours following injection of tuberculin (2 c. c.) on 76th day.	Negative	Excess clear fluid in peritoneal cavity; few hard nodules, one caseous, in omentum; bronchial glands enlarged; otherwise normal.
D7	15 minutes at 60°	250	380	Died in 15 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Moderate enlargement of inguinal glands; peritoneal fluid excessive; caseous nodules in omentum and caseous abscess between spleen and kidney; small caseous abscess at site of inoculation; bronchial glands slightly enlarged.

						Secondary guinea pig inoculated intraperitoneally with emulsion of lesions of D7 remained well, did not respond to 2 c. c. tuberculin on the 71st day, gained weight; no lesions at autopsy.
D8	18 minutes at 60°	250	....	Died on 5th day	do	Death due to peritonitis resulting from intestinal perforation.
D9	20 minutes at 60°	250	500	Died in 7 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Slight excess clear fluid in peritoneal cavity; omentum contains a caseous nodule; perisplenitis; otherwise normal. *Secondary guinea pig* (see D7), negative.
D10	23 minutes at 60°	250	550	Chloroformed 76th day	do	Seemed sick as a result of tuberculin injection, but recovered; no lesions at autopsy.
D11	25 minutes at 60°	250	205	Died on 15th day	do	Omentum contains few small nodules, not caseous; slight enlargement of inguinal, axillary, and mediastinal glands; all else normal.
D12	28 minutes at 60°	250	475	Died in 6 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Not emaciated; caseous nodule at site; excess clear fluid in peritoneum; few small nodules in omentum; otherwise normal.
D13	30 minutes at 60°	250	440	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Caseous nodule in pancreas and omentum; nodule on peritoneal surface of diaphragm. *Secondary guinea pig* (same details as D7), negative.
D14	35 minutes at 60°	255	500	Chloroformed 76th day	do	Became sick following tuberculin reaction, but recovered; nothing abnormal at autopsy excepting a few grayish patches on spleen not resembling tubercles. *Secondary guinea pig* (same details as D7), negative.
D15	40 minutes at 60°	260	460	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Omental pancreas thickened and contains caseous lumps, some 3 mm. in diameter; patches of perisplenitis and one perinephritis. *Secondary guinea pig* (same details as D7), negative.
D16	45 minutes at 60°	265	430	Died in 16 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Caseous nodules in pancreas and omentum; caseous abscess over right kidney; small hard nodule on diaphragm. *Secondary guinea pig* (same details as D7), negative.
D17	50 minutes at 60°	265	520	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Omental pancreas enlarged and nodular; one ileolumbar gland and inguinal glands enlarged, but not caseous; whitish area on edge of spleen; otherwise normal. *Secondary guinea pig* (same details as D7), negative.
D18	60 minutes at 60°	265	500	Chloroformed 76th day	do	Failed to react to subcutaneous injection of 2 c. c. tuberculin; nothing abnormal found at autopsy.

TABLE NO. 5.—*Series E.*

Bovine culture, Bovine III, from Department of Agriculture.

Twenty cubic centimeters special market milk rubbed up with culture.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, few in clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk contained 2,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

The temperature during this experiment averaged from 64.8° to 65.1° C.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig. Initial.	Weight in grams of guinea pig. Final.	Time and mode of death.	Results.	Remarks.
E1	Control, 24.5° C. (at start)	265	235	Died on 15th day	Generalized tuberculosis.	Emaciation; large caseous mass each testicle; caseous nodules in seminal vesicles, generalized miliary tuberculosis of peritoneum; omentum rolled up and contains many hard yellow nodules, some size of pea; spleen enlarged; anterior mediastinal gland caseous; inguinal glands enlarged.
E2	...do	270	250	...do	Generalized tuberculosis (?).	No autopsy.
E3	60° (took 2¼ minutes to heat from 24.5° to 60°).	270	240	Died on 23d day	Generalized tuberculosis.	Inguinal and axillary glands enlarged; great numbers of tubercles scattered over peritoneal surface; masses and nodules in omentum and near site, spleen and both lungs contain tubercles.
E4	65° (took 5 minutes to heat from 24.5° to 65°).	270	450	Chloroformed 77th day	Negative	Did not react to subcutaneous injection of 2 c. c. tuberculin; well nourished; caseous abscess in abdominal wall and at site; many large caseous areas in omentum; mesenteric glands enlarged, anterior mediastinal slightly; pregnant. *A secondary guinea pig* inoculated intraperitoneally with an emulsion of lesions and glands of E4 remained well, gained weight, failed to respond to 2 c. c. tuberculin on the 68th day, autopsy negative.

E5	2 minutes at 65°	280	350	Died in 5 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in diaphragm and omentum; excess of peritoneal fluid. *Secondary guinea pig*, negative.
E6	3 minutes at 65°	280	400	Died in 17 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in omentum; same in region of each ovary; hard, noncaseous nodules on under surface of diaphragm; bronchial glands enlarged. *Secondary guinea pig*, negative.
E7	5 minutes at 65°	285	195	Died in 12 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Omentum reduced to cheesy sac; large nodule on diaphragm; bronchial glands enlarged; few small yellow patches in liver and one in spleen. *Secondary guinea pig*, negative.
E8	8 minutes at 65°	285	480	Died in 13 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Nothing abnormal found at autopsy. Secondary guinea pig (same as E4), negative.
E9	10 minutes at 65°	290	445	do	do	Caseous nodule in omentum; otherwise normal. *Secondary guinea pig*, negative.
E10	12 minutes at 65°	290	425	Chloroformed 77th day	do	Did not react to subcutaneous injection of 2 c. c. tuberculin; nodules on diaphragm, pleura; mediastinal glands enlarged, not caseous; omentum thickened, but no nodules; no caseation except glandular enlargements; no definite findings. *Secondary guinea pig*, negative.
E11	15 minutes at 65°	300	490	Died in 10 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous abscess at site; caseous nodules in omentum and between liver and intestine. *Secondary guinea pig* died in 7 days from septic peritonitis; no evidence of tuberculosis.
E12	20 minutes at 65°	260	400	Died in 13 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in omentum, 1 in spleen, and between spleen and kidney, and between kidney and liver; all else normal. *Secondary guinea pig*, negative.

TABLE No. 6.—*Series F.*

Bovine culture, B I, from Trudeau, Saranac Laboratory.

This culture was obtained from a cheesy mass in the apex of left lung of a cow reacting to tuberculin test at 41.1° C. on September 28, 1902; age of cow, 18 to 20 years.

Some of the caseous material inoculated into guinea pigs and the culture recovered from the inguinal glands of the guinea pigs on Löffler's sheep serum, subculture on cow serum.

A moderately heavy suspension was rubbed up in a sample of mixed market milk.

Upon smears some bacilli were found in each field, lying singly and in clusters.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
F1	Control; 28° C. (at start)	265	315	Died in 5 hours following injection of tuberculin (2 c. c.) on 59th day.	Generalized tuberculosis.	Emaciation and very extensive generalized tuberculosis of all organs, except lungs; inguinal glands caseous.
F2	..do..	250	450	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	.....do.....	Fairly well preserved; pronounced generalized tuberculosis; 1 inguinal gland caseous; abscess at site; spleen and omentum contain many tubercles, also liver; mediastinal and hepatic glands caseous; areas in lung perhaps tuberculous.
F3	60° (took 4 minutes to heat from 28° to 60°).	280	475	Died in 5 hours following injection of tuberculin (2 c. c.) on 59th day.	.....do.....	Inguinal glands enlarged and caseous; axillary glands enlarged; caseous nodule at site; spleen very large and filled with tubercles; omentum has numerous hard and caseous nodules; tubercles in liver; anterior mediastinal gland enlarged, also bronchial glands which are caseous.

F4	1 minute at 60°	280	500	Died in 20 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well-preserved caseous abscess in abdominal wall at site; few small, hard caseous nodules in omentum; tubercles in spleen, which is bound down; liver contains disseminated tubercles; bronchial glands enlarged and caseous.
F5	2 minutes at 60°	275	440	Died in 19 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Omentum contains hard nodules, not caseous; tubercles on spleen; few nodules on surface of liver; bronchial glands enlarged.
F6	3 minutes at 60°	290	475	Chloroformed 60th day	Negative	Did not react to tuberculin injection (2 c. c.) subcutaneously; normal at autopsy except small yellow nodule on spleen.
F7	4 minutes at 60°	270	430	Died in 14 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well nourished; hard subsplenic nodule; omentum nodular; no caseation; nothing else abnormal.
F8	5 minutes at 60°	250	465	do	do	Few hard nodules in omentum, none caseous; otherwise normal.
F9	7 minutes at 60°	280	445	Chloroformed 60th day	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; few hard caseous nodules in omentum; otherwise normal.
F10	10 minutes at 60°	320	510	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; 1 small hard nodule in omentum; no caseation; otherwise normal.
F11	15 minutes at 60°	335	400	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; 1 large caseous nodule in omentum.
F12	20 minutes at 60°	280	480	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; hard nodules in omentum; no caseation; perisplenitis; otherwise normal.
F13	30 minutes at 60°	310	550	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well nourished; small caseous abscess in abdominal wall and at site; small patch perisplenitis; few nodules in omentum, one caseous; bronchial glands enlarged.
F14	40 minutes at 60°	270	525	Chloroformed 60th day	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; well preserved, nothing abnormal found at autopsy.
F15	50 minutes at 60°	265	440	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well preserved; caseous nodule about the size of a pea in right seminal vesicle; several hard and caseous tubercles in omentum; spleen adherent; 1 mesenteric gland caseous; otherwise normal.

TABLE NO. 7.—*Series G.*

Bovine culture, H Ray, from the Rockefeller Institute for Medical Research.

This culture was kindly sent me by Dr. Flexner, who states that it was isolated from the mesenteric gland of a cow.

A heavy suspension rubbed up in a small quantity of mixed market milk.

Stained smears showed numerous bacilli in each field and also numerous clumps of various sizes.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41.0; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final			
G1	Control; 27° C. (at start)	310	265	Died on 18th day	Generalized tuberculosis.	Emaciation; generalized miliary tuberculosis; deposit upon peritoneum and tunica vaginalis; omentum rolled up and contains many large and small nodules; spleen enlarged and tuberculous; anterior mediastinal glands caseous; caseous abscess at site in abdominal wall; femoral glands enlarged.
G2	....do	315	340	Died on 29th day	....do	Emaciation; caseous abscess in abdominal wall at site; superficial glands enlarged; peritoneum studded with nodules, contains sanious fluid; omentum one large hard and caseous mass; tubercles in spleen and lungs; mediastinal glands enlarged.
G3	60° (took 4½ minutes to heat from 27° to 60°).	270	550	Died in 6 hours following injection of tuberculin (2 c. c.) on 63d day.	Localized tuberculosis.	Well preserved; omentum contains few hard and caseous nodules; ileolumbar gland size of pea and caseous; mesenteric glands enlarged, not softened; glands and celiac axis enlarged and size of filbert, caseous.
G4	1 minute at 60°	290	400	Died on 44th day	Negative	Streptococcal double pneumonia; well preserved; one caseous gland in axilla; few hard nodules in omentum; one retroperitoneal gland caseous; otherwise normal.

G5	2 minutes at 60°	320	495	Died in 6 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Omentum contains few small nodules, one caseous; ileolumbar glands enlarged and caseous; glands in region of celiac axis enlarged and caseous; few mesenteric glands enlarged, but not caseous.
G6	3 minutes at 60°	280	375	Died on 31st day	do	Double streptococcal pneumonia; well preserved; no lesions indicating tuberculosis at autopsy.
G7	4 minutes at 60°	265	575	Died in 19 hours following injection of tuberculin (2 c. c.) on 65th day.	do	No indications of tuberculosis at autopsy.
G8	5 minutes at 60°	260	225	Died on 16th day	do	Do.
G9	7 minutes at 60°	325	285	Died on 14th day	do	Double pneumonia (streptococcal); few whitish firm noncaseous nodules posteriorly under peritoneum.
G10	10 minutes at 60°	290	450	Died in 18 hours following injection of tuberculin (2 c. c.) on 64th day.	do	Left inguinal glands enlarged to size of marble and caseous; few hard nodules in omentum.
G11	15 minutes at 60°	320	480	Died in 11 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Few nodules in omentum; whitish area in spleen; otherwise normal. *Secondary guinea pig* inoculated intraperitoneally with emulsion of lesions of G11 remained well, gained weight, did not respond to 2 c. c. tuberculin on the 65th day; no lesions at autopsy.
G12	20 minutes at 60°	275	350	Died on 50th day	do	Abscess of heart, which contained a large mass occupying whole lower half, yellowish, fluctuating; cocci and bacilli in lymph nodes and spleen smears negative for tubercle bacilli; no evidence of tuberculosis.
G13	30 minutes at 60°	280	490	Died in 13 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Omentum nodular; anterior mediastinal glands enlarged on right side, not caseous; perisplenitis. *Secondary guinea pig* inoculated intraperitoneally with emulsion of lesions; of G13 remained well, gained weight, did not respond to 2 c. c. tuberculin; no lesions at autopsy.
G14	40 minutes at 60°	265	450	Chloroformed 65th day	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; small caseous nodule in abdominal wall at site of inoculation; inguinal glands slightly enlarged; perisplenitis; otherwise normal.
G15	50 minutes at 60°	330	550	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; pregnant; abortion; large deep yellow patch on liver; all else normal.

TABLE NO. 8.—*Series II.*

Bovine culture, H Ray, from the Rockefeller Institute for Medical Research.

This culture was kindly sent me by Dr. Flexner, who states that it was isolated from the mesenteric gland of a cow.

A heavy suspension rubbed up in a small quantity of mixed market milk.

Stained smears showed numerous bacilli in each field and also numerous clumps of various sizes.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
H1	Control; 31° C. (at start)	250	265	Died on 38th day	Generalized tuberculosis.	Emaciation; caseous nodule at site; inguinal and axillary glands enlarged; peritoneum studded with tubercles; spleen enlarged with tubercles; omentum one large hard caseous mass; liver full of tubercles; lungs contains few nodules; mediastinal glands enlarged.
H2	do	290	210	Died on 35th day	do	Much emaciation; caseous mass at site; inguinal glands enlarged; peritoneum studded with miliary tubercles; liver and spleen enlarged with advanced tuberculosis; omentum large hard caseous mass; tubercles in lungs.
H3	60° (took 5 minutes to heat from 31° to 60°).	255	410	Died in 8 hours following injection of tuberculin (2 c. c.) on 63d day.	Negative	Hard noncaseous nodes in omentum; otherwise normal.
H4	1 minute at 60°	315	440	Chloroformed 64th day	do	Did not die as result of subcutaneous injection 2 c. c. tuberculin; noncaseous node in omentum; otherwise normal.
H5	3 minutes at 60°	290	410	do	do	Did not react to subcutaneous injection 2 c. c. tuberculin; omentum somewhat shrunken; otherwise normal throughout.

H6	....do....	320	520	Died in 8 hours following injection of tuberculin (2 c. c.) on 63d day.	.....do	Nothing abnormal found at autopsy.
H7	4 minutes at 60°	290	470	Died in 14 hours following injection of tuberculin (2c.c.) on 63d day.	.....do	Cheesy nodules in omentum; all else normal.
H8	5 minutes at 60°	260	320	Died in 10 hours following injection of tuberculin (2 c.c.) on 63d day.	.....do	Omentum somewhat nodular; no other evidence of tuberculosis. *A secondary guinea pig* inoculated with an emulsion of the lesions from H_8 remained normal, gained weight; no evidence of tuberculosis at autopsy; chloro formed 30th day on account of submixillary abscess (streptococci).
H9	7 minutes at 60°	255	350	.....do	.....do	Few nodules in omentum; anterior mediastinal glands enlarged; no other lesions.
H10	10 minutes at 60°	305	440	Chloroformed 64th day	.....do	Did not react to subcutaneous injection 2 c. c. tuberculin; few hard nodes in omentum; otherwise normal.
H11	15 minutes at 60°	270	485	.....do	.....do	Did not react to subcutaneous injection 2 c. c. tuberculin; no lesions.
H12	20 minutes at 60°	265	450	...do	.....do	Did not react to subcutaneous injection 2 c. c. tuberculin; few nodes in omentum; spleen bound down by extensive adhesions; liver dotted with fine yellow spots; slight enlargement of ileolumbar glands; otherwise normal; pregnant. *Secondary guinea pig* inoculated peritoneally with lesion and organs of H_{12} gained weight; did not respond to tuberculin on 64th day; no lesions at autopsy.
H13	30 minutes at 60°	290	430	Died in 13 hours following injection of tuberculin (2c.c.) on 63d day.	.....do	Few small nodes in omentum; perisplènitis; anterior mediastinal glands enlarged.
H14	40 minutes at 60°	310	455	Chloroformed 64th day	.....do	Did not react to subcutaneous injection 2 c. c. tuberculin; few small omental nodes; otherwise normal throughout; pregnant.
H15	50 minutes at 60°	260	420	Died in 10 hours following injection of tuberculin (2 c. c.) on 63d day.	.....do	Omentum slightly thickened, but not nodular; spleen adherent posteriorly; no evidence of tuberculosis.

TABLE NO. 9.—*Series J.*

Bovine Culture, Bovine III, from Department of Agriculture.

Ten-day old culture of glycerin potassium biphosphate broth. The entire pellicle from a 100 c. c. Erlenmeyer flask rubbed up with 15 c. c. of a special (certified) milk.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, also stray clumps and clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk had a reaction of 0.153 per cent acidity and contained 9,000 bacteria (other than tubercle bacilli) just before it was used for this experiment and 20 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
J1	Control; 25° C. (at start)	280	200	Died on 20th day	Generalized tuberculosis.	Emaciation; inguinal glands enlarged; small caseous abscess at site; miliary tubercles studded over peritoneum; tubercles in liver, spleen, lungs, omentum rolled up into hard fibrous and caseous mass.
J2	do	275	225	do	do	Emaciation; inguinal and axillary glands enlarged, small node at site; miliary tubercles on peritoneum; tubercles in liver, spleen, mediastinal glands, and lungs; omentum rolled up into hard fibrous and caseous mass.
J3	do	270	200	Died on 19th day	do	Emaciation; axillary glands enlarged; large caseous abscess at site; miliary tubercles on peritoneum; tubercles in liver, spleen, and lungs.
J4	59.7° (took 5 minutes to heat from 25° to 59.7°).	285	290	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Emaciation; inguinal glands enlarged; caseous abscess in abdominal wall at site; omentum contains large caseous nodules; tubercles in spleen, which is enlarged and bound down; liver, peritoneum, ileolumbar glands, celiac group, mesentery, anterior mediastinal glands, and lungs contain nodes.
J5	60° (took 6 minutes to heat from 25° to 60°).	260	305	Died in 8 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Caseous nodules at site; large caseous areas in spleen, liver, and omentum; anterior mediastinal, celiac group, and retroperitoneal glands enlarged.

J6	1 minute at 60	270	335	Died in 4 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Right inguinal gland enlarged and caseous; few small nodules on peritoneum; tubercles in spleen, liver, omentum (caseous), ileolumbar glands, celiac group (size of marble and caseous), mesenteric nodes, anterior mediastinal glands, and lungs.
J7	2 minutes at 60°	270	360	Died in 8 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Inguinal gland somewhat enlarged, red; nodules upon surface of diaphragm, in omentum and spleen; surface plaques on spleen and liver; mesenteric and anterior mediastinal glands enlarged.
J8	3 minutes at 60°	265	360	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Small caseous nodule at site; nodules in omentum, some caseous; tubercles in spleen (enlarged), one on edge of liver; lumbar glands, celiac group, mesenteric and mediastinal glands enlarged.
J9	4 minutes at 60°	265	305	Chloroformed 37th day	Negative	Perisplenitis; omentum rolled up and contains many caseous nodules; no definite glandular enlargement; liver has numerous fine light areas, not like tubercles; otherwise normal.
J10	5 minutes at 60°	285	390	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Omentum contains nodes, some caseous; spleen bound down, otherwise normal; liver contains many tubercles (?); celiac group enlarged, not caseous; lumbar glands caseous; mesenteric nodes enlarged.
J11	6 minutes at 60°	250	350	Died in 10 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Small caseous nodule at site; noncaseous nodule in omentum; few yellow areas in substance of spleen and liver; ileolumbar and celiac group enlarged; otherwise normal.
J12	8 minutes at 60°	280	335	Died in 13 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Nodule on under surface of diaphragm and in omentum, not caseous; patches perisplenitis and hepattis; retroperitoneal glands enlarged; otherwise normal.
J13	10 minutes at 60°	290	420	Died in 10 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Nodules on under surface of diaphragm; caseous tubercles in omentum; surface patches on spleen; retroperitoneal, celiac group, and anterior mediastinal glands enlarged.
J14	12 minutes at 60°	250	360	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Few small nodules, not caseous, in omentum; lumbar glands slightly enlarged; spleen bound down posteriorly; one anterior mediastinal gland slightly enlarged; otherwise normal.
J15	15 minutes at 60°	260	270	Chloroformed 37th day	do	Did not react to subcutaneous injection 2 c. c. tuberculin; nothing abnormal at autopsy.
J16	20 minutes at 60°	250	305	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Caseous abscess at site in abdominal wall; few small nodules, 1 caseous, in omentum; perisplenitis; liver bound to anterior abdominal wall; otherwise normal.

Conclusion.—From these experiments it is evident that the tubercle bacillus in milk loses its infective properties for guinea pigs when heated to 60° C. and maintained at that temperature for twenty minutes, or to 65° C. for a much shorter time.

It should be remembered that the milk in these tests was very heavily infected with virulent cultures, indicated by the prompt deaths of the control animals. Milk would practically never contain such an enormous amount of infection under natural conditions. It is justifiable to assume that if 60° C. for twenty minutes is sufficient to destroy the infectiveness of such milk when injected into the peritoneal cavity of a guinea pig, any ordinary market milk after such treatment would be quite safe for human use by the mouth so far as tubercle bacilli are concerned.

THE EFFECTS OF DEAD TUBERCLE BACILLI.

In the foregoing work endeavor was made to distinguish the lesions produced by the dead tubercle bacilli. For comparison a number of animals were inoculated with milk containing dead tubercle bacilli (killed at 100° C.). The necropsy notes of this series, given in Table No. 10, are very instructive, especially when compared with the foregoing work (Tables 1–9).

Prudden and Hodenphyl [a] found "that tubercle bacilli which have been killed by boiling or otherwise, when introduced into the body of the rabbit either beneath the skin or into the serous cavities, or into the blood vessels and the air spaces of the lungs, are capable, as they slowly disintegrate, of stimulating the cells of the tissues where they lodge to proliferation, and to the production of new tissue morphologically similar to tubercle tissue in its various phases. Necrosis of the new-formed cells may occur, but this differs in some respects from the coagulation necrosis induced under the usual conditions. Dead tubercle bacilli are also markedly chemotactic and capable of causing local suppuration and abscess."[b]

James Miller found that[c] "Dead tubercle bacilli can, then, produce changes which in their early stages are the same as those produced by the living organism. They differ in acuteness only. When these dead bacilli are introduced into the body they attract the leucocytes of both varieties. The cells take up the organism, the polymorphonuclear forms degenerate; the mononuclear cells are, however, capable of further development, and, probably with the assistance of connective tissue cells, form the epithelioid and giant cells. Inclosed within their cell prison the bacilli undergo a gradual disintegration, setting free a poison which may produce partial death of a cell, or, if in sufficient quantity, may produce the death of many cells. There is not the same activity in the leucocytes, nor the same vacuolation of the protoplasm and development of pseudopodia in the mononuclear forms. The epithelioid cells have not the same size or the same branched appearance, the giant cells are more rounded and smooth. The whole process gives one the impression of less activity. In the later stages the difference is more marked. There is not the same wide distribution and extension of the process, nor is there the same constant addition of new phagocytes at the margin which will become epithelioid cells and help to swell the giant cell. There is, however, in these later stages the same impression of a partial cell necrosis. The bacilli disappear, but the cells persist and show the peculiar hyaline appearance. Further, there is the same polymorphonuclear leucocytosis in the last stage of pseudo-tubercle that we have seen in true tubercle."

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[a] For further details concerning the effects of dead tubercle bacilli in the body see Prudden & Hodenphyl, N. Y. Med. Journ., June 6 and 20, 1891, and Prudden, ibid, Dec. 5, 1891. For summary of later work, with bibliography, see Herxheimer, Ziegler's Beitr., vol. 33, p. 363; also, Miller, Journ. Path. and Bact., vol. 10, 1905, p. 351.

[b] Delafield, Francis, and Prudden, T. Mitchell: A Text-book of Pathology, 8th ed. New York, Wm. Wood & Co., 1907. 1057 p. Illus. 8°.

[c] Miller, James: "The Histogenesis of the Tubercle" Journal of Pathology and Bacteriology, 1905, vol. 10, pp. 1–48.

In my own experiments, in order to avoid the confusion resulting from the effects produced by dead tubercle bacilli, doubtful lesions were carried over into another animal. These "secondary" guinea pigs appear in Tables Nos. 1 to 9. The method used was as follows:

The lesions, and sometimes the organs, were ground up in a mortar with salt solution, strained through gauze, and the extract thus obtained injected into the peritoneal cavity of a young normal guinea pig. Only two of these secondary guinea pigs developed generalized tuberculosis. The others remained well, gained weight, failed to respond to tuberculin, and gave no evidence of an active tuberculosis at necropsy. Some of these secondary animals had small tuberclelike masses in the omentum, due to absorption of the products introduced into the peritoneal cavity.

Almost all the animals were tested with tuberculin in the hope that this test might distinguish between the lesions of live and of dead tubercle bacilli. This, however, was found not to be the case, for when the eight animals with lesions caused by tubercle bacilli known to be dead, were injected with 2 c. c. of tuberculin (O. T.) (see Table No. 10), three of them died. The series of guinea pigs in this table is instructive further in that it illustrates that dead tubercle bacilli in milk, when injected into the peritoneal cavity, may produce very extensive tubercular lesions with coagulation necroses (caseation), etc.

TABLE No. 10.—*Results of injecting dead tubercle bacilli in milk into the peritoneal cavity of guinea pigs.*

No. of guinea pig.	Date of inoculation.	Tubercle bacilli in milk heated to—	Culture.	Weight in grams.		Subcutaneous inoculation 2 c. c. tuberculin.		Date and manner of death.	Necropsy notes.
				Initial.	Final.	Date.	Result.		
D19	Sept. 12, 1907	100° C. 1 hour...	Bovine III...	270	325	Nov. 7, 1907	Sick; recovered.......	Chloroformed Nov. 8, 1907..	Moderate hemorrhagic local reaction at site of tuberculin inoculation; no edema; hard nodules in omentum, not caseous; perisplenitis; ileolumbar and inguinal glands slightly enlarged.
D20	.....do......	100° 1 hour......	.....do........	265	490	Nov. 12, 1907	.....do...............	Chloroformed Nov. 13, 1907.	Very marked hemorrhagic and edematous reaction at site of tuberculin inoculation; no glandular enlargements; few hard nodules in omentum; pregnant.
F16	Sept. 9, 1907	100° 30 minutes.	Bl Saranac...	260	500	Nov. 7, 1907	No effect...........	Chloroformed Nov. 8, 1907 .	Enlarged mesenteric glands, not caseous.
G16	.....do......	100° 10 minutes.	II Rav, Rockefeller Institute.	280	465	Oct. 25, 1907	Sick; recovered.......	Chloroformed Oct. 26, 1907..	Edema and hemorrhagic reaction at site of tuberculin inoculation; fine miliary nodules like tubercles on peritoneum; liver mottled with fine tubercle-like areas; omentum contains nodules, not caseous.
J17	Oct. 7, 1907	100° 1 hour.....	Bovine III...	255	355	Nov. 12, 1907	Died in 8 hours......	....	Emaciation; hemorrhagic and edematous reaction at site of tuberculin inoculation; inguinal glands slightly enlarged and reddened; caseous nodule in abdominal wall at site of inoculation; peritoneum smooth; excess fibro-serous fluid in peritoneal cavity; omentum rolled up into large tuberculous mass containing numerous caseous foci; perisplenitis; cœliac

									group of glands enlarged, not caseous; mesenteric nodes enlarged; numerous whitish nodules on under surface of diaphragm; anterior mediastinal glands enlarged and caseous.
J18	do	do	do	255	375	do	Died in 10 hours		Moderate hemorrhagic and edematous reaction at site of tuberculin inoculation; excessive fluid in peritoneal cavity; omentum contains few small caseous nodules; spleen bound down; otherwise normal.
J19	do	do	do	280	375	do	No effect	Chloroformed Nov. 13, 1907	Marked hemorrhagic local reaction; no glandular enlargements; nodules on under surface of diaphragm and in omentum; liver and spleen contain tubercle-like areas.
J20	do	do	do	250	350	do	Died in 12 hours		Hemorrhagic and edematous reaction at site of tuberculin inoculation; inguinal glands, both sides, enlarged and reddened; axillary glands less so; excessive clear fluid in peritoneal cavity; tendency to coagulate; small caseous nodule in abdominal wall at site; omentum rolled up and contains numerous nodules, some caseous; spleen bound down; caseous mass size of pea between spleen and kidney; spleen enlarged and contains four nodules on surface; ileolumbar glands slightly enlarged; under surface of diaphragm roughened with numerous whitish nodules; anterior mediastinal glands enlarged and caseous.

REVIEW OF THE WORK OF OTHERS UPON THE THERMAL DEATH POINT OF THE TUBERCLE BACILLUS.

The early work upon the thermal death point of the tubercle bacillus is clouded in the obscurity of meager data and insufficiently controlled and imperfectly oriented experiments.

Martin,[a] 1882, concludes, from two experiments with fragments of lung of a tuberculous guinea pig, that with an exposure to a moist temperature of 80° C. the infectivity of tubercle bacilli is not lost.

May,[b] 1883, undertook to determine how long the milk of tuberculous cows must be boiled to render it surely innocuous. He experimented with injections of material into the peritoneal cavity of guinea pigs. All the injections of cooked fluids, milk, etc., gave negative results. Most of the experiments with raw milk of tuberculous cows also gave negative results; a few positive. He concludes that there is small danger of infection from raw milk, and none at all from cooked milk, for human beings.

Sormani,[c] 1884, is reported by De Man to have injected milk containing tubercle bacilli into guinea pigs after having heated it ten minutes at 70°, 80°, and 90° C., with the result that all the animals became tuberculous. When, however, the milk was kept seething for five minutes, cooled, and injected, the animals remained sound.

Schill and Fischer,[d] 1884, studied the action of a temperature of 100° C. upon tuberculous sputum. They found that 100° of dry temperature did not surely destroy the virulence of tuberculous sputum whether dry or moist.

Voelsch[e] concluded that boiling did not destroy the virulence in tuberculous products from rabbits, serum cultures, or tuberculous sputum. His experiments were made upon rabbits, and it appears that he misinterpreted the lesions produced by dead tubercle bacilli. He went so far as to believe that boiling twice, while it attenuates, does not destroy the virulence of the tubercle bacillus.

Yersin,[f] 1888, tested the thermal death point of tubercle bacilli "with spores and without spores." The tubercle bacilli "without spores" were obtained from spleen pulp of a rabbit dead of the intravenous injection of a culture. Some of this spleen pulp, very rich in bacilli, was drawn into capillary tubes hermetically sealed and plunged into a water bath kept at the desired temperature. After heating, the pulp was inoculated upon glycerine bouillon. Tubes heated to 55° for ten minutes gave subsequent growth; those heated to 60° for ten minutes, no growth; those heated to 70° for ten minutes, no growth. The tubercle bacilli "with spores" came from an old culture in glycerin bouillon. Tubes heated to 55° for ten minutes gave subsequent growth; those heated to 60° for ten minutes gave subsequent growth; those heated to 65° for ten minutes, no growth; those heated to 70° for 10 minutes, no growth.

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[a] Martin, Hippolyte: Sur la transformation du tubercule vrai ou infectieux en corps étranger inerte sous l'influence de hautes températures et de réactifs divers. Rev. de méd., vol. 2, 1882, p. 913–927.

[b] May, F.: Ueber die Infectiosität der Milch perlsüchtiger Kühe. Arch. f. Hyg., vol. 1, 1883, p. 121.

[c] Sormani: Annali universali di med., vol. 269, 1884.

[d] Schill and Fischer: Ueber die Desinfection des Auswurfs der Phtisiker. Mittheil. a. d. k. Gesndh., vol. 2, 1884, p. 131.

[e] Voelsch: Beiträge zur Frage nach der Tenacität der Tuberkelbacillen. Ziegler & Nauwerk's Beiträge zur path. Anat. u. Phys., vol. 2, 1887.

[f] Yersin, A.: De l'action de quelques antiseptiques et de la chaleur sur le bacille de la tuberculose. Ann. de l'Inst. Pasteur, vol. 2, 1888, p. 60.

Yersin concludes that the bacillus of tuberculosis resists a temperature of 60° C. for ten minutes, and that it is remarkable that the resistance of the "spores" to the heat is not greater than that of the bacilli themselves. He made no tests upon animals.

He found, contrary to the work of Schill and Fischer, that tuberculous sputum heated to 100° for two minutes is always sterile.

Thus early in the history of these investigations the idea became prevalent that the tubercle bacillus possessed a resistance to heat considerably greater than that of other nonspore-bearing pathogens. Indeed, at this time the tubercle bacillus was regarded by many as occurring in a spore-bearing form, and some of the writers spend much time in telling whether the strains they used were spore-bearing or not, omitting more pertinent facts.

Bitter,[a] 1890, accepted the results of Yersin to the effect that the tubercle bacillus when evenly distributed through milk is killed by an exposure to 75° C. for ten minutes, and inquires if a lower temperature, desirable for practical reasons, might not be equally efficacious. He cautiously descends as far as 68° to 69°, and finds that milk to which infected sputum had been added and placed in test tubes immersed in a previously warmed water bath is rendered innocuous to guinea pigs inoculated intraperitoneally, if the exposure has lasted as much as twenty minutes. Controls injected with the unheated milk died of tuberculosis in five weeks. To be perfectly safe, the author recommends that the exposure to 68°–69° be increased to thirty minutes.

Bang,[b] 1891, states that his experiments demonstrate that heating to 85° C., and sometimes even to 80° C., kills all the tubercle bacilli; that heating for five minutes to 70° C., sometimes to 60° C., so enfeebles the virus that he believes it is no longer able to infect when fed by way of the digestive tract. Ten years later Bang was obliged to correct in part some of his early results.

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[a] Bitter, H.: Versuche über das Pasteurisiren der Milch. Zeit. f. Hyg., vol. 8, 1890, p. 240–286.

[b] Bang: Le danger supposé de la consommation du lait et de la viande sains en apparence mais provenent d'animaux atteints de la tuberculose. Trans. internat. Cong. hyg. et démog., 1890, p. 193. Also: Experimentelle Untersuchungen über tuberculöse Milch. Deut. Zeit. f. Tiermed., vol. 17, 1891, p. 1.

Many observers nowadays would regard these results as indicating an unduly high resistance of the organism and would suspect that Bang gave the tubercle bacillus a handicap in three ways: (1) In using pathological milk prone to form little clots on heating, which protect the tubercle bacilli (see Barthel and Stenström); (2) in not avoiding the formation of the protective surface pellicle (see Th. Smith, Russell and Hastings, Hesse), and (3) possibly in failing to differentiate the lesions caused by dead tubercle from those caused by living germs (Smith, Miller, Prudden, and Hodenphyl).

Bonhoff,[a] 1892, did not work with milk, but gives some data obtained with pure cultures of tubercle bacilli in liquid media, which may be accepted as contributory evidence. Young cultures, the ultimate antecedents of which are not stated, were exposed in test tubes placed in a water-bath, the temperature being observed on a thermometer placed inside the tube. Guinea pigs were inoculated intraperitoneally before and after exposure. The significant findings are, that while 50° for sixty minutes failed to kill the bacteria, 60° for twenty minutes did kill them. Exposures of less than twenty minutes were not made. The author unfortunately had some difficulty in preventing the development of lung tuberculosis in his animals. For instance, the pigs receiving the material heated to 60° for forty minutes, 70° for sixty minutes, and 80° momentarily, developed what he calls and is at considerable pains to differentiate as *inhalation* tuberculosis.

Grancher and Ledoux-Lebard,[b] 1892, appreciated the occurrence of lesions produced by dead tubercle bacilli in their inoculated animals. They showed that human tubercle bacilli are more sensitive to the action of temperatures of 50° C. than are avian tubercle bacilli. Heating the avian bacilli to 50° or 60° for fifteen minutes did not kill them. Animals inoculated died of tuberculosis. However, the organisms heated to 60° were attenuated. Heating to 70° under the same conditions noted was sufficient to destroy the power of the avian tubercle bacilli to infect laboratory animals.

They heated cultures of human tubercle bacilli suspended in distilled water and found that five minutes at 60° sufficed to attenuate the virulence. Heating to 60° for ten minutes and twenty minutes also greatly attenuated the virulence. When heated to 70° C. for one, two, five, or ten minutes the tubercle bacilli were killed.

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[a] Bonhoff: Die Einwirkung höherer Wärmegrade auf Tuberkelbacillen-Reinkulturen. Hyg. Runds., vol. 2, 1892, p. 1009.

[b] Grancher, J., and Ledoux-Lebard: Tuberculose aviaire et humaine. Arch. de méd. expér. et d'anat. pathol., vol. 4, 1892, p. 1.

Forster,[a] 1892, sealed off small glass tubes containing for the most part the milklike material squeezed from the cut surface of tubercular udders and placed these in a water bath kept at the desired temperature for various periods. They were then cooled quickly and injected intraperitoneally into guinea pigs. The presence or absence of living tubercle bacilli was determined by autopsy, by the lesions, or by carrying the suspect material over into other animals. The bacilli appeared not to be killed by three hours exposure to 55°, forty-five minutes exposure to 60°, or momentary exposure to 80°, but were killed by six hours exposure to 55° (sputum), one hour exposure to 60° and 80°, momentary exposure to 95°. These figures indicate a high degree of resistance.

It is difficult to explain the high figures obtained by Forster, especially in the light of the work of Smith, Russell and Hastings and my own.

The next year the same author,[b] reviewing the situation, emphasizes that pasteurizing apparatus is of two kinds; (1) in which the milk passes over heated surfaces and is momentarily raised to a given temperature, and (2) in which the milk is heated in kettles, bottles, etc., and may be kept for a certain time at the desired temperature. He says that the latter are the only ones applicable to milk containing tubercle bacilli, because momentary heating to temperatures insufficient to change the taste of the milk does not kill tubercle bacilli.

De Man,[c] 1893, working under Forster, obtained results long current in text-books and which have been accepted as the standards for practical work in many regulations for dairy methods. The following brief summary of early work on the subject culled by him from Galtier (1879) shows the discrepancies and inadequacies of this period—faults, however, which continue to appear in recent work:

Author.	Temperature.	Time.	Result.
Chaveau and Arloing	70°, 100°	30 minutes	No certainty of killing tubercle bacilli.
Touissaint	73°, 76°, 80°	(?)	Virulence remains.
Gerlach	100°	30 minutes	Virulence remains, but weakened.
Martin	80°, 100°	(?)	Virus withstands this heat.
Klebs			Boiled milk may cause tuberculosis.
Frerichs, Parrot, and Martin			Boiled tubercular sputum innocuous.
Vallin			Paper impregnated with tubercle bacilli loses virulence by dipping in boiling water.

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[a] Forster, J.: Ueber die Einwirkung hoher Temperaturen auf Tuberkelbacillen. Hyg. Runds., vol. 2, 1892, p. 869.

[b] Forster, J.: Ueber die Einwirkung hoher Temperaturen auf Tuberkelbacillen. Hyg. Runds., vol. 3, 1893, p. 669.

[c] De Man, C.: Über die Einwirkung von hoher Temperaturen auf Tuberkelbacillen. Arch. f. Hyg., vol. 18, 1893, p. 133.

De Man states that few, if any, of these authors recite the conditions and methods of their experiments. He used material from the substance scraped from the cut surface of tuberculous udders, and in some instances tubercular sputum. The work was done in the same manner as Forster's, q. v., and is apparently a part of or a continuation of the same series of experiments. His results may be condensed as follows:

Tubercle bacilli are killed by the following temperatures: In udder juice, 55° after four hours exposure, 60° after one hour exposure (not after forty-five minutes), 80° after five minutes exposure, 90° after two minutes exposure, 95° after one minute exposure; in sputum, 65° after fifteen minutes exposure, 70° after ten minutes exposure. Exposure for less time failed to kill. Control animals injected with unheated material became tubercular.

Smith,[a] 1899, says, in criticism of this work, that such dense suspensions can not be used to determine the thermal death point to be compared with those of other bacilli, nor can they be regarded as imitating the conditions under which tubercle bacilli occur in milk. It might be added that De Man's work at two very interesting temperatures (65° and 70°) was performed with sputum and not even udder juice.

Schroeder,[b] 1894, obtained what may be regarded as an early hint that comparatively low temperatures may be effective in killing the tubercle bacillus in milk. With most other observers at that time, he had been led to regard the tubercle bacillus as a nonspore-bearing pathogen almost unique among this class in its resistance to heat. He acted upon the advice of Yersin who, at the suggestion of Roux, attempted to make use of this property to rid milk of those other organisms which frequently produce disease or death when injected into animals. Schroeder, attempting to discover the presence of tubercle bacilli in market milk, heated the specimens to 60° for fifteen minutes before injection. Fortunately, he controlled this procedure with milk artificially inoculated with tubercle bacilli, and the failure to get tuberculosis in market milk specimens was paralleled with a similar failure in the controls. The inference was that 60° for fifteen minutes could, under some circumstances, kill tubercle bacilli.

Woodhead,[c] 1895, obtained very discordant results, which it is difficult to interpret. It is probable that they are explained, in the case of experiments conducted with milk artificially contaminated with pieces of tubercular tissue, by the fact that clumps of considerable size (too large to pass through the inoculating needle and requiring glass pipettes for inoculation) were added to the milk, and that these would in some manner protect the bacilli; and in the case of naturally tuberculous milk by the probability of protection by coagula—especially prone to occur in the concentrated pathological product—and by the pellicle which forms on the surface of heated milk.

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[a] Smith, Theobald: The thermal death point of tubercle bacilli in milk and other fluids. Journ. Exper. Med., vol. 4, 1899, p. 217.

[b] Schroeder, E. C.: Further experimental observations on the presence of tubercle bacilli in the milk of cows. U. S. Bur. An. Industry, Bull. 7, 1894, p. 75.

[c] Woodhead, G. Sims: Report of the Royal Commission appointed to inquire into the effect of food derived from tuberculous animals on human health. Parts 8, 9, 12, and 13. 1895.

Considering the inoculation experiments with both the natural and the artificial tuberculous milk, the following minimum lethal temperature exposures are found in the tables: 50° C. killed surely only after fifteen hours; 55°, after seven hours; 60°, after eight hours; 65°, after four hours;[a] 70°, after forty-five minutes; 75°, after thirty minutes; 80°, —; 85°, after two and one-half minutes; 90° did not kill.

This summary shows how Woodhead's results contradict themselves and differ from those of other observers. When the results with the naturally tuberculous milk alone are considered, the results accord more with Bang's; for example: 60° kills surely after forty-five minutes; 65°, after two and one-half minutes; 70°, after two and one-half minutes.

Marshal,[b] 1899, first sterilized milk, then added tuberculous material from cows, injected control animals with this suspension, and then exposed the milk in shallow dishes in a "pail sterilizer" placed on a water bath. Heat was applied to the water bath until the temperature in all parts of the vapor chamber was 68°. This temperature was maintained twenty minutes. The shallow layer of milk was devised to secure equal temperature throughout the milk. The controls died in a few weeks of tuberculosis, but none of the test animals killed and examined at the same time showed any signs of tuberculosis. He states that from other experience he knows that 60° for ten minutes will not kill tubercle bacilli, but does not detail the experiments upon which this conclusion is based.

Theobald Smith,[c] 1899, contributed an article to the literature of this subject which marks an era in the history of the pasteurization of milk based upon the thermal death point of the tubercle bacillus. Smith's experiments were most carefully performed. Many pitfalls and errors which some of his predecessors had fallen into were avoided and pointed out. The only possible objection to the work, which applies with equal force to all laboratory experiments, is that it was not done on a scale of practical dairy pasteurization.

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[a] No experiment between three and four hours; killed after two hours.

[b] Marshal, Charles E.: Killing the tubercle bacillus in milk. Michigan State Agricultural College experiment station, Bull. 173, 1899.

[c] Smith, Th.: The thermal death point of tubercle bacilli in milk and some other fluids. Journ. Exper. Med., vol. 4, 1899, p. 219.

He limited his investigation to the effects of 60° upon artificially infected milk and demonstrated by a series of experiments, which should be consulted in the original to appreciate their full force and value, that an exposure to 60° for fifteen minutes is enough to kill tubercle bacilli suspended in distilled water, normal salt solution, bouillon and milk, provided that in the case of milk the production of a surface pellicle is prevented. The pellicle which forms during the exposure may contain living tubercle bacilli after sixty minutes exposure at 60° C.

Smith concluded that (1) tubercle bacilli when suspended in distilled water, normal salt solution, bouillon and milk are destroyed at 60° C. in fifteen to twenty minutes (the larger number are destroyed in five to fifteen minutes); (2) when tubercle bacilli are suspended in milk the pellicle which forms during the exposure at 60° C. may contain living bacilli after sixty minutes. He also states that these experiments demonstrate that tubercle bacilli are no more resistant to heat than many other bacilli not producing spores.

Forster and De Man, had they used a homogeneous suspension in normal milk, must have arrived at similar results with their sealed tubes, where a pellicle does not form; but as Barthel and Stenström have shown, the pathological material from tuberculous udders, probably because of its abnormal reaction, is especially prone upon heating to the formation of little clumps of proteid coagula, which exert a protective influence against heat upon the tubercle bacilli.

Morgenroth,[a] 1900, apparently had not the benefit of Smith's work when he did his own experiments. He determined that 70° for ten minutes or 100° momentarily did not kill the tubercle bacilli in milk from tuberculous udders. Scum formation was not mentioned and the minutiæ of the experiments are omitted. He worked with the "thermophore," an apparatus which maintains a heat of 55° or more for several hours, and says that artificially inoculated milk is freed from living tubercle bacilli in this apparatus after three hours, two hours being insufficient.

Kobrak,[b] 1900, states that the thermophore kills tubercle bacilli in milk infected with sputum in four, but not in three, hours. The temperature ranged probably somewhat over 50° during this time.

Beck,[c] 1900, concludes from his experiments that raising milk to the boiling point is insufficient to kill the contained tubercle bacilli, but that they are surely killed by three minutes boiling. He also found that when sterilized milk infected with fine suspensions of tubercle bacilli was exposed to 70° for ten minutes, 70° for thirty minutes, and 80° for thirty minutes, tuberculosis resulted in inoculated guinea pigs.

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[a] Morgenroth: Versuche über Abtödtung von Tuberkelbacillen in Milch. Hyg. Runds., vol. 10, 1900, p. 685.

[b] Kobrak: Die Bedeutung des Milch-Thermophores für die Säuglingsernährung. Zeit. f. Hyg., 1900, vol. 34, p. 518.

[c] Beck: Experimentelle Beiträge zur Untersuchungen über die Marktmilch. Deut. Viertelj. f. off. Gesundhpfl., vol. 32, 1900, p. 430.

Galtier,[a] 1900, found that five to six minutes heating of milk to 70° C., and even 85° C., was not sufficient to destroy the tubercle bacillus when that product is richly contaminated. He prepared an emulsion from the spleens and lungs of tuberculous rabbits, which was mixed up with the milk. He concludes from his studies that milk richly contaminated by the addition of tuberculous matter is not surely sterilized at 85° for six minutes. He recommends boiling as the only safe procedure to practice.

Rabinowitsch,[b] 1900, made the statement that recent investigations at the Institut für Infektionskrankheiten in Berlin had shown that tubercle bacilli in milk are surely killed only by a temperature of 100° C. Her results recalled attention to the fact that the tubercle bacillus, like all other organisms embedded in fat, is more resistant to heat, and that this may account for the irregular results obtained by investigators.

Russell and Hastings,[c] 1900, made a valuable contribution to our knowledge of pasteurization, inasmuch as they applied the principles elucidated by Smith to work upon a practical scale. They made use of a Potts' pasteurizer, which represents that type of machine which permits of heating milk for a specified time at a specified temperature in a close chamber, thorough distribution of the heat being secured by the rotary movements of the machine. Bovine cultures of determined virulence were scraped from dog-serum slants rubbed up with a 0.6 per cent salt solution, sedimented to remove large clumps, and then the supernatant suspension was added in definite proportions to the milk. This infected milk, after control animals had been inoculated, was placed in small glass tubes closed with a cork and rubber guard, and these tubes were then heated in a water bath up to the desired temperature and then dropped into the apparatus already raised to that point. During the exposure the temperature of the milk fluctuated no more than 1 degree and that only momentarily. The only temperature employed was 60° C. After a specified time the tubes were removed and the contents injected intraperitoneally into guinea pigs. The results are clearly shown in the following tables:

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[a] Galtier, V.: Le lait tuberculeux cesse-t-il d'être dangereux après un court chauffage à 70–75 degrés? Compt. rend. des seances et mém. de la soc. de biol., vol. 2, 2d ser., 1900, p. 120.

[b] Rabinowitsch, Lydia: Deut. med. Woch., vol. 26, 1900, p. 491.

[c] Russell, H. L., and Hastings, E. G.: Thermal death point of tubercle bacilli under commercial conditions. University of Wisconsin Agricultural Experiment Station, 17th Ann. Rep., 1900.

Time exposed.	Result in each series.				
	I.	II.	III.	IV.	V.
60°, 45 minutes	– –	....	....	..	....
60°, 30 minutes	– –	....	....	..	....
60°, 20 minutes	....	– –	....		– –
60°, 15 minutes	– –	– –	....	.	– –
60°, 10 minutes	....	– –	– –	– –	– –
60°, 5 minutes	....	....	+ +	+ +	....
Control	+	+	+	+	+

– = No tuberculosis.
+ = Tuberculosis.
Two pigs in each experiment.

A series was then run which shows the difference in effect of heating in open vessels and in sealed or closed tubes, the latter being placed in Potts' machine.

Time exposed.	Series VI.		Series VII.	
	Potts' sealed tube.	Open bottle.	Potts' sealed tube.	Open bottle.
60°, 15 minutes	....		..	+ +
60°, 10 minutes	– –	+ +		+ +
60°, 5 minutes	+ +	+ +	+ +	...
Control	+	+	+	......

– = No tuberculosis.
+ = Tuberculosis.
Two pigs in each experiment.

In their experiments, milk heated at 60 degrees began to show visible signs of pellicle after about twenty minutes, but the authors think that flocculent patches exist before this. For practical work they advise pasteurization at 60 degrees for twenty minutes in closed apparatus.

Herr,[a] 1901, worked with cream only. Here the problem is different from that of milk pasteurization. It is not necessary to avoid those temperatures which change the taste of the product, which is used for making butter, and hence a higher temperature and very brief exposure may be employed, permitting the use of the so-called continuous pasteurizers which operate with a great economy of time and fuel. In Herr's experiments cream was shaken up with tuberculous sputum, the mixture sealed off in tubes of 4 c. c. capacity, leaving as little air as possible in the tubes. He had previously determined how long it would take to heat the contents of such tubes to certain temperatures by dropping them into boiling water. The infected tubes were therefore placed in boiling water for the time necessary to heat to the desired temperature and then placed in a water bath maintained at this temperature. After the lapse of the desired time they were cooled rapidly and injected into guinea pigs intraperitoneally. The results are shown in the table, + and – indicating that the animals became tuberculous or not. The results were controlled by examination after the death or killing of the animals.

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[a] Herr: Das Pasteurisiren des Rahmes als Schutz gegen die Verbreitung der Tuberkelbacillen durch Butter. Zeit. f. Hyg., vol. 38, 1901, p. 182.

Temperature and time.	Result.	Temperature and time.	Result.
65°, 15 minutes	–	80°, 3 minutes	–
68°, 15 minutes	–	85°, 5 seconds	–
70°, 7 minutes		85°, 5 minutes	–
72°, 7 minutes		89°, 30 seconds	–
75°, 3 minutes	–	90°, 5 seconds	–
75°, 5 minutes	[a]	95°, 5 seconds	–
80°, 5 seconds	+		

[a] Pseudo-tubercle, Pfeiffer.

As the most practical temperature and exposure, Herr recommends 85° for two minutes for cream from which butter is to be made. This is obtained in the "continuous" form of apparatus.

Hesse,[a] 1901, corroborates Smith's findings. Sterilized milk inoculated with a culture of human tubercle bacilli several weeks old was sealed off in small tubes and these dropped into a Pfund's sterilizer previously heated. After twenty minutes exposure they were taken out and cooled and the contents injected into guinea pigs. The animals were killed after fifty-five days. Controls receiving unheated milk had high-grade generalized tuberculosis. Pigs receiving milk heated at 57° for twenty minutes had less severe lesions; those receiving milk heated at 58° for twenty minutes had still severe lesions, and one receiving milk heated at 60° for twenty minutes was entirely sound, the other having a small translucent nodule in the diaphragm, in which no tubercle bacilli were found.

The experiments corroborate Smith's findings. The gradation of severity of lesions inversely with the increased temperature is interesting.

Levy and Bruns,[b] 1901, showed that a temperature varying between 65° and 70° C. and operating for fifteen to twenty-five minutes effectually killed bovine and human tubercle bacilli in milk, when 1 liter of the artificially infected milk was exposed in a d'Arsonval thermostat—i. e., under conditions where a pellicle would not be formed. The work is interesting especially because a liter of milk was used in the experiments. It is to be regretted that they did not experiment with lower temperatures.

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[a] Hesse: Ueber die Abtödtung der Tuberkelbacillen in 60° C. warmer Milch. Zeit. Tiermed., 1901, vol. 5, p. 5–6.

[b] Levy, E., and Bruns, Hugo: Ueber die Abtödtung der Tuberkelbacillen in der Milch durch Einwirkung von Temperaturen unter 100°. Hyg. Runds., vol. 11, 1901, p. 669.

Barthel and Stenström,[a] 1901, report some preliminary work which must be interpreted as showing the influence of coagula in protecting the tubercle bacilli from the action of heat. This clot formation was probably due to the abnormal reaction of the pathological fluid from tuberculous udders employed. Animals injected with this material became tuberculous after it had been heated to 75° for fifteen minutes and 80° for ten minutes. Temperatures and exposures sufficient to kill the tubercle bacilli under the conditions of the experiments were not employed. In this connection the subsequent work of these authors should be consulted (vide infra).

Bang,[b] 1902, reported a large number of experiments which give results at variance with those of this author's former investigations. To avoid the formation of the surface pellicle and foam, in some of his work he exposed the milk in closed metallic boxes containing a thermometer immersed in a water bath and kept constantly in motion during the exposure to insure an equal distribution of the heat. As in former work, he used rabbits to test the virulence of exposed material by the intraperitoneal method. He experimented with pure cultures of the bacillus, noting whether growth was delayed or inhibited by exposure to heat, and further determined the effect of feeding the heated milk to rabbits and swine. The essential features of his results (under conditions which prevent the formation of scum) are as follows:

Temperature and exposure.	Result.
60°, 15 minutes	Prevented tuberculosis in 2 animals, but 2 others became slightly tuberculous.
65°, 1 to 15 minutes	Effectually prevented tuberculosis.
70°, 75°, 80°, and 85°, momentarily	Do.

Tuberculous milk from diseased udders was used in some of these experiments.

Rabbits receiving one 30 c. c. feeding of milk exposed for two minutes to 60°, 65°, 70°, 75°, 80°, and 85° remained free from tubercle, although controls fed with raw milk became tuberculous.

Swine fed with 150 c. c. each of tuberculous milk heated for half a minute at 60° became tuberculous, while those receiving milk heated to 70°, 75°, and 80° for half a minute remained sound.

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[a] Barthel, Chr., and Stenström, O.: Beitrag zur Frage des Einflusses hoher Temperaturen auf Tuberkelbacillen. Cent. f. Bakt., vol. 30, 1901, p. 429.

[b] Bang: Ueber die Abtödtung der Tuberkelbacillen bei Wärme. Zeit. f. Tiermed., vol. 6, 1902, p. 81.

From these results Bang concludes that while heating at 60° for fifteen minutes does not prevent peritoneal infection it weakens the bacteria so that they are innocuous by way of the alimentary tract.

Concerning his work with cultures, it is interesting to note that glycerin-bouillon tubes sowed with particles of growth from a young culture failed to develop any growth after exposure to 60° and 65° for five minutes, although unheated controls grew well.

Bang did not report results at 60° for twenty minutes.

Tjaden,[a] 1903, obtained very high thermal death points for the tubercle bacillus and concludes that 85° for one to two minutes is necessary to kill that organism. Bang attributes this high resistance to the coagula which formed during some of the experiments.

Rullmann,[b] 1903, in a preliminary note, says that he can not agree with those observers who state that 60° for fifteen or twenty minutes kills the tubercle bacilli in milk. He found that milk infected with tuberculous sputum exposed to 65° for half an hour "with all precautions" still caused tuberculosis when injected intraperitoneally into guinea pigs. He promises a full report, which, however, we have not been able to obtain.

Barthel and Stenström,[c] 1904, continue their experiments relative to the effect of the reaction of milk upon the resistance to heat of the contained tubercle bacilli. They divided into two portions some tuberculous milk; one portion was rendered abnormally acid, another neutral in reaction. The neutral milk withstood 90° without coagulation; the acid milk coagulated at 60°.

Results with 80°: One minute exposure killed in uncoagulated milk, but not in coagulated, i. e., acid milk.

At 85°: Momentary exposure failed to kill in either. One minute exposure killed in uncoagulated but not in coagulated milk.

While these experiments are very limited in scope, they at least suggest a source of error in the work of those who used the secretion of tubercular udders in their experiments. Such material is prone to be abnormal in reaction, and protective clots may have been formed.

Sydney Phillips,[d] 1904, mentions merely that the results obtained by Russell and Hastings[e] were confirmed by Macfadyean of the Lister Institute.

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[a] Tjaden: Abtödtung der pathogenen Keime in der Molkereimilch durch Erhitzung ohne Schâdigung der Milch und Milchproducte. Deut. Med. Woch., 1903, p. 976.

[b] Rullman: Ueber die Abtödtung von Tuberkelbacillen in erhitzer Milch. Münch. med. Woch., 1903, vol. 31, p. 1342.

[c] Barthel, Chr., and Stenström, O.: Weitere Beiträge zur Frage des Einflusses hoher Temperaturen auf Tuberkelbacillen in der Milch. Cent. f. Bakt., vol. 37, 1904, p. 459.

[d] Phillips, Sydney: The milk supply of a hospital; American method of sterilization. The Hospital, July 16, 1904.

[e] Russell and Hastings. University of Wisconsin Agricultural Experiment Station, 17th Ann. Rep., 1900.

Russell and Hastings,[a] 1904, had worked previously from the standpoint of a comparatively low temperature, 60° C. They now worked from the standpoint of a short exposure, such, for instance, as is available in a continuous-action pasteurizing machine, to determine how low a temperature could be safely used with such necessarily short exposures. Milk infected with human and bovine cultures of known origin and virulence was exposed in sealed glass tubes placed in a water bath. It took three-fourths of a minute for the contained milk to reach the temperature of the outside water. Virulence was determined upon guinea pigs by the intraperitoneal method.

Their results show that material exposed to 160° F. (71.1° C.) for one minute was freed of living tubercle bacilli. This applied to a very virulent bovine strain as well as to less virulent bovine and human strains. The authors conclude that 71.1° C. or more for a period of one minute suffices to destroy the virulence of bovine tubercle cultures so that the disease is not produced in guinea pigs inoculated with amounts of the cultures ranging from 2 to 5 milligrams. As definite amounts of the culture had been added to the milk, the dosage of each animal could be approximated. It is fair to note that the virulent bovine strain produced lesions in animals receiving the heated milk, which, however, were justly regarded by the authors as being due to dead bacteria, since animals inoculated with this material failed to develop tuberculosis. The authors do not unreservedly recommend this temperature and exposure for routine practical pasteurization without further investigation under all conditions.

Schwellengroebel,[b] 1904, shows that when milk is pasteurized in bottles the tubercle bacilli, if present, may be protected against the heat by (1) the rough or cracked surface of the rubber washers used by some dairymen in closing the bottles, (2) the dried layer of milk in improperly cleansed bottles, (3) the pellicle formed on the surface of heated milk, and (4) the foam formed in the same way. The remedies are, of course, sterilization of bottles, washers, etc., and heating under conditions which prevent the formation of foam and pellicle.

Zelenski,[c] 1906, is a recent advocate of higher temperatures in the pasteurization of milk. He finds that 76° for ten, fifteen, or twenty minutes is insufficient to kill the bacilli.

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[a] Russell, H. L., and Hastings, E. G.: Effects of short periods of exposure to heat on tubercle bacilli in milk. University of Wisconsin Agricultural Experiment Station, 21st Ann. Rep., 1904, p. 178.

[b] Schwellengröbel, M.: Ueber das Pasteurisiren der Milch. Cent. f. Bakt., Abt. 2, vol. 12, 1904, p. 440.

[c] Zelenski: Zur Frage der Pasteurisation der Säuglingsmilch. Jahrb. f. Kinderheilkunde, 1906, vol. 63, 3, p. 288.

It is difficult, if not impossible, to briefly summarize the above work. The following table necessarily leaves out many factors:

TABLE No. 12.—*Showing the thermal death point of the tubercle bacillus as found by various investigators.*

Investigator.	Killed at—	Not killed at—
Martin, 1882		80°.
May, 1883	By cooking	
Sormani, 1884	Boiling, 5 minutes.	90° for 10 minutes.
Schill and Fisher, 1884		100°.
Voelsch, 1887		100°, boiling twice.
Yersin, 1888	60°, 10 minutes (−spores) 60°, 10 minutes (+spores)	
Bitter, 1890	68°, 20 minutes	
Bang, 1891	70°, 5 minutes (enfeebles) 60°, 5 minutes (sometimes enfeebles) 80° (sometimes kills) 85° (always kills)	
Bonhoff, 1892	60°, 20 minutes	50°, 60 minutes.
Grancher and Ledeux-Lebard, 1892	60°, 5 minutes (attenuates) 70°, 1 minute (kills)	
Forster, 1892	60°, 6 hours 95°, momentary	55°, 3 hours. 60°, 45 minutes. 80°, momentary.
De Man, 1893	55°, 4 hours 60°, 1 hour	60°, 45 minutes.
Schroeder, 1894	60°, 15 minutes	
Woodhead, 1895	50°, 15 hours 60°, 8 hours 60°, 45 minutes 70°, 45 minutes 70°, 2½ minutes	90° (results contradictory).
Marshall, 1899	68°, 20 minutes	60°, 10 minutes.
Th. Smith, 1899	60°, 15 to 20 minutes	
Morgenroth, 1900	55°, 3 hours	70°, 10 minutes. 100°, momentary.
Kobrak, 1900	50°, 4 hours	
Beck, 1900	100°, 3 hours.	100° 80°, 30 minutes.
Galtier, 1900		85°, 6 minutes.
Russell and Hastings, 1900	60°, 20 minutes	
Herr, 1901	65°, 15 minutes	80°, 5 seconds.
Hesse, 1901	60°, 20 minutes	
Levy and Bruns, 1901	65°, 15 minutes	
Barthel and Stenström, 1901		70°, 15 minutes.
Bang, 1902		60°, 15 minutes.
Tjaden, 1903	85°, 1 to 2 minutes	
Rullmann, 1903	65°, 30 minutes	60°, 20 minutes.
Barthel and Stenström, 1904	80°, 1 minute (uncoagulated)	80°, 1 minute (coagulated).
Russell and Hastings, 1904	71°, 1 minute	
Zelenski, 1906		76°, 20 minutes.
Rosenau, 1907	60°, 20 minutes	

The above tabular statement shows that my results agree with the work of Yersin, Bonhoff, Schroeder, Th. Smith, Russell and Hastings, and Hesse in that 60° for twenty minutes is sufficient to kill the tubercle bacillus.

BACILLUS TYPHOSUS.

REVIEW OF THE WORK OF OTHERS.

Sternberg,[a] 1888, found that the temperature of 56° C. (138.8° F.) for ten minutes killed the Bacillus *typhi abdominalis*. Sternberg adopted ten minutes as the standard time of exposure for his experimental work upon the thermal death point of a long list of micro-organisms. He took pains to insure uniform temperature by personal attention and frequent stirring of the water bath with a glass rod. The micro-organisms were first drawn into hermetically sealed capillary tubes with expanded extremities. The tubes were immersed in a water bath, maintained at the desired temperature, for the standard time, namely, ten minutes. No attempt was made to fix the thermal death point within narrower limits than 2° C.

Pfuhl,[b] 1888, placed a number of test tubes containing a bouillon culture of typhoid bacilli with spores (?) in water baths at 50° and 60° C. for ten, twenty, thirty, and fifty minutes. All the 50° cultures retained life and motility. In the 60° bath only those exposed ten minutes regularly showed movement and growth.

Van Geuns,[c] 1889, determined the effect upon bacteria of momentary heating to temperatures varying from 50° to 80° C. and immediate cooling. He used four methods:

Method 1: Stock-broth culture of the organism was introduced into 10 per cent gelatin tubes with a platinum loop. Some of these inoculated tubes were incubated as controls and the others placed in a water bath kept at a constant temperature. The control with thermometer in a gelatin tube showed that it took ninety seconds to reach 80° C. and that it took about the same time to cool the tube in tap water to 12° C. After heating, the gelatin tubes were incubated at 20°. This was the first method used by Van Geuns for a study of the common organisms in milk.

For special purposes he adopted one of the following methods:

Method 2: Capillary pipettes were filled with broth cultures. The capillary tube was inserted into a cork along with a thermometer and both immersed into a test tube of water, which in turn rested in a water bath at boiling temperature. As soon as the required temperature in the tube was reached it was removed from the bath and cooled. The capillary tube was then broken, and, with a needle, stabs were made in gelatin.

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[a] Sternberg, Manual of Bacteriology, 1892, p. 146, and Report of the Committee on Disinfectants of the American Public Health Association. 1888, pp. 136 and 152 (p. 140).

[b] Pfuhl: Zur Sporenbildung der Typhusbacillen. Cent. f. Bakt., vol. 4, 1888, p.771.

[c] Van Geuns: Über das Pasteurisiren von Bakterien. Ein Beiträgé zur Biologie der Microorganismen. Arch. f. Hyg., vol. 9, 1889, p. 369.

Method 3: This is a modification of method No. 1. The test tube, containing broth or water, with a thermometer and capillary tube, is put into a water bath kept at temperature of the tube tested, or 3 to 5 degrees higher.

Method 4: Capillary tubes and the thermometer placed in a small Erlenmyer flask, which can be well shaken. The fluid in the flask was accurately maintained at a constant temperature by placing the flask in a small water bath, which, in turn, was placed in a larger one and a thermoregulator used to control the temperature of the outer bath.

Van Geuns concluded that the thermal death points based on the above experiments were as follows:

Time required to reach temperature stated.	Cholera. (° C.)	Typhoid. (° C.)
1 minute	59	60
5 minutes	54	56

Lazarus,[a] 1890, used 20 liters of raw market milk divided into small flasks; these he inoculated with cholera and typhoid. This infected milk was then run through a small Thiel's continuous pasteurizer at the rate of 1 liter per forty seconds. The temperatures were made to vary from 60° to 96° C. in the different experiments. At intervals of one to two minutes samples of one-half cubic centimeter were taken and plated on fluid gelatin. At 70° C. living typhoid and cholera were found, but none at 75° C. These results are more a test of the efficiency of this particular pasteurizer than an accurate determination of the thermal death points of the micro-organisms used.

Janowski,[b] 1890, inoculated typhoid bacilli from a four-day potato growth into gelatin after it reached the temperature to be tested. At the end of five or ten minutes roll tubes were made. He found those exposed 57° for five minutes always remained sterile. Sometimes he got a growth at 56° for five minutes, and once at 56° for ten minutes. The bacilli were not put into the gelatin until it reached the temperature to be tested, and the work therefore does not exactly imitate the conditions of pasteurization.

Neisser,[c] 1895, used the same methods as von Geuns. The temperature was taken from a control tube and the time counted from the moment the temperature reached the desired degree. Some typhoid bacilli resisted three minutes' heating at 60° to 61.75° C., but fifteen minutes' heating at this temperature killed all.

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[a] Lazarus, A.: Die gebräuchlicheren Mittel zur Conservirung der Milch. Zeit. f. Hyg., vol. 8, 1890, p. 235.

[b] Janowski: Zur Biologie der Typhusbacillen. Cent. f. Bakt., vol. 8, 1890, p. 167.

[c] Neisser, Max: Dampf-Desinfection und Sterilisation von Brunnen und Bohrlöchern. Zeit. f. Hyg., vol. 20, 1905, p. 308.

Hesse,[a] 1900, obtained sterile milk directly from the cow by the use of special precautions in test tubes, and inoculated these tubes with cultures of typhoid, cholera, diphtheria, and plague. They were heated to 60° C. for fifteen to twenty minutes in a water bath and plated. The plates remained sterile. Controls gave a good growth. No record is given of the effect of lower temperatures or shorter times. The possible effects of the "germicidal" power of the raw milk was not considered.

Bassenge,[b] 1903, made about fifty-two determinations of the effect of various temperatures upon typhoid in raw milk. He used large quantities of raw milk—one-half to 1½ liters—to which had been added a broth culture tube of typhoid. The work was done in iron, porcelain, and enamel-ware vessels, over gas and coal flames of varying intensity. The milk was raised from room temperature to the desired point. Bassenge found that when milk was raised to a temperature of 60° C. in from five to ten minutes and plates made on Conradi-Drigalski medium no typhoid was found. When, however, the milk was raised to 60° C. in three minutes, typhoid colonies were present. He does not indicate whether the degrees reported were top or bottom temperatures. His figures varied slightly, depending upon the composition of the vessel in which the milk was heated.

Friedel, Kutscher, and Meinicke,[c] 1904, working in Koch's laboratory under Kolle's direction, found that the typhoid, paratyphoid, and enteritidis group of bacilli have the same resistance to heat. They were all killed without exception when the temperature of the milk reached 59° C., provided ten minutes elapsed from the beginning of the heating until this temperature was reached.

These experiments were made by previously heating one-half to 1 liter of milk to 100° in an Erlenmeyer flask and after this cooled inoculating it with 10 c. c. of a fresh twenty-four hour bouillon culture. The flasks were then set in a large kettle of hot water and precautions taken to obtain an equable distribution of heat. It required ten minutes to heat this quantity of milk from 15° to 60° C. At intervals of one minute 5 c. c. were taken with a sterile pipette and added to 50 c. c. of bouillon, incubated twenty-four hours, and then plated upon agar.

A review of the literature upon the viability of the typhoid bacillus, with especial reference to its thermal death point, will be found in Hyg. Rundschau, vol. 14, 1904, p. 353, by Ina Rosquit, and Zeit. f. Hyg., vol. 20, 1895, p. 308, by Max Neisser.

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[a] Hesse, W.: Ueber das Verhalten pathogenen Mikroorganismen in pasteurisirter Milch. Zeit. f. Hyg., vol. 34, 1900, p. 346.

[b] Bassenge, R.: Ueber das Verhalten der Typhusbazillen in der Milch und deren Produkten. Deut. med. Woch., vol. 29, 1903, p. 264.

[c] Friedel, Kutscher, and Meinicke: Die Widerstandfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 13, 1904–5, p. 324.

ORIGINAL EXPERIMENTS.

In the following work I used nine different strains of typhoid bacilli. The results were as follows:

Experiment No. 1.

B. typhosus.

Forty-eight hours' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	Loop of milk culture in bouillon.
"Dawson" culture; reaction, 0.216 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	(a)	Do.
"P. I." culture; reaction, 0.207 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	(a)	Growth.[b]
"P. I." culture; reaction, 0.198 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	(a)	Do.
"McF." culture; reaction, 0.225 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.[c]
4 minutes after immersion	60	No growth.
5 minutes after immersion	(a)	Do.
"Smith" culture; reaction, 0.198 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	(a)	Do.
"Hay" culture; reaction, 0.234 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	(a)	Do.
"Stock" culture; reaction, 0.171 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	(a)	Do.

[a] One minute at 60°.

[b] Contaminated.

[c] Delayed.

EXPERIMENT No. 1—Continued.

Milk culture.	Temperature (°C.).	Loop of milk culture in boullion.
"Chicago" culture; reaction, 0.225 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	[a]	Do.

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[a] One minute at 60°.

EXPERIMENT No. 2.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in sterilized milk. Reaction, 0.28 per cent acid.

The milk at 31.5° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	Loop of milk culture in boullon.	Number of colonies per loop in agar.
At start	31.5	Growth	70,000
1½ minutes after immersion	55	do	25,000
1¾ minutes after immersion	56		
1⅘ minutes after immersion	57	Growth	10,140
2¼ minutes after immersion	58	do	1,780
2½ minutes after immersion	59	do	121
3¾ minutes after immersion	60	No growth	1
4¾ minutes after immersion	1 minute at 60	do	None.
5¾ minutes after immersion	2 minutes at 60	do	None.
5¾ minutes after immersion	3 minutes at 60	do	None.
6¾ minutes after immersion	4 minutes at 60	do	None.
7¾ minutes after immersion	5 minutes at 60	do	None.

EXPERIMENT No. 3.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in sterilized milk. Reaction, 0.198 per cent acid.

Milk at 29° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	29	Growth	[a]
1½ minutes after immersion	55	do	24
2¼ minutes after immersion	58	No growth	Lost.
4 minutes after immersion	60	do	None.
5 minutes after immersion	1 minute at 60	do	None.

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[a] Innumerable.

EXPERIMENT No. 4.

B. typhosus.

Culture, "Hopkins;" twenty-four hours' growth in sterilized milk. Reaction, 0.27 per cent acid.

The milk at 27° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	27	Growth	[a] 40,000
1 minute after immersion	50	do	[a] 40,800
1½ minutes after immersion	55	do	[a] 40,600
2¼ minutes after immersion	58	do	2,400
2¾ minutes after immersion	59	do	360
4 minutes after immersion	60	do	12
6 minutes after immersion	2 minutes at 60	No growth	None.
8 minutes after immersion	4 minutes at 60	do	None.
10 minutes after immersion	6 minutes at 60	do	None.
12 minutes after immersion	8 minutes at 60	do	None.
14 minutes after immersion	10 minutes at 60	do	None.

[a] About.

EXPERIMENT No. 5.

B. typhosus.

Twenty-four hours' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
"Stock" culture; reaction, 0.162 per cent acid:		
At start	28	Growth.
2½ minutes after immersion	58	No growth.
3 minutes after immersion	59	Do.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	Do.
6 minutes after immersion	2 minutes at 60	Do.
"Hay" culture; reaction, 0.126 per cent acid:		
At start	28	Growth.
2½ minutes after immersion	58	No growth.
3 minutes after immersion	59	Do.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	Do.
6 minutes after immersion	2 minutes at 60	Do.

Experiment No. 6.

B. typhosus.

Culture, "Hay;" forty-eight hours' growth in sterilized milk. Reaction, 0.162 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	Do.
4¼ minutes after immersion	1 minute at 60	No growth.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

Experiment No. 7.

B. typhosus.

Culture, "Chicago;" forty-eight hours' growth in sterilized milk. Reaction, 0.171 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2⅓ minutes after immersion	58	Do.
2¾ minutes after immersion	60	Do.
3¾ minutes after immersion	1 minute at 60	No growth.
4¾ minutes after immersion	2 minutes at 60	Do.
5¾ minutes after immersion	3 minutes at 60	Do.
6¾ minutes after immersion	4 minutes at 60	Do.
7¾ minutes after immersion	5 minutes at 60	Do.

Experiment No. 8.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2⅓ minutes after immersion	58	Do.
2¾ minutes after immersion	60	Do.
3¼ minutes after immersion	1 minute at 60	Do.

EXPERIMENT No. 8—Continued.

Milk culture.	Temperature (°C.).	⅓ c. c. milk culture in bouillon.
4¾ minutes after immersion	2 minutes at 60	No growth.
5¾ minutes after immersion	3 minutes at 60	Do.
6¾ minutes after immersion	4 minutes at 60	Do.
7¾ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT No. 9.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1⅓ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	No growth.
4¼ minutes after immersion	1 minute at 60	Do.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT No. 10.

B. typhosus.

Culture, "Smith;" forty-eight hours' growth in sterilized milk. Reaction, 0.162 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1⅓ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	Do.
4¼ minutes after immersion	1 minute at 60	No growth.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT No. 11.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in whole milk that had been sterilized by the fractional process three days. Reaction, 0.252 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2¼ minutes after immersion	57	Do.
3 minutes after immersion	59	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	No growth.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	Do.
14¼ minutes after immersion	10 minutes at 60	Do.

EXPERIMENT No. 12.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in whole milk that had been sterilized by the fractional process three days. Reaction, 0.288 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.)	1 c. c. milk culture in bouillon.
At start	21	Growth.
1½ minutes after immersion	55	Do.
2 minutes after immersion	57	Do.
2½ minutes after immersion	59	Do.
3⅔ minutes after immersion	60	Do.
4⅔ minutes after immersion	1 minute at 60	No growth.
5⅔ minutes after immersion	2 minutes at 60	Do.
6⅔ minutes after immersion	3 minutes at 60	Do.
8⅔ minutes after immersion	5 minutes at 60	Do.
13⅔ minutes after immersion	10 minutes at 60	Do.

Conclusion.—The evidence is plain that milk heated to 60° C. and maintained at that point for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C. and few survive to 60° C.

BACILLUS DIPHTHERIÆ.

REVIEW OF THE WORK OF OTHERS.

Welch and Abbott,[a] 1891, tested the thermal death point of the diphtheria bacillus upon bouillon cultures and suspensions, in sterlized salt solution, in capillary glass tubes, and found that the bacillus is killed at a temperature of 58° C. in ten minutes. Details not stated.

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[a] Welch and Abbott: The etiology of diphtheria. Johns Hopkins Hospital Bull., vol. 2, 1892.

Sternberg,[a] 1892, quotes Löffler to the effect that the thermal death point of the bacillus of diphtheria is 60° C.

ORIGINAL EXPERIMENTS.

In my work I used Park's bacillus No. 8, which is a strong toxine producer and virulent for laboratory animals. My results follow:

EXPERIMENT NO. 13.

B. diphtheriæ.

Culture, "Park, No. 8;" twenty-four hours growth in sterilized milk. Reaction of milk, 0.108 per cent acid.

The milk at 32° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in glycerin agar.
At start	32	Growth	1,240
2 minutes after immersion	50	No growth	None.
2½ minutes after immersion	57	do	None.
3¾ minutes after immersion	59	do	None.
4½ minutes after immersion	60	do	None.
5½ minutes after immersion	1 minute at 60	do	None.

EXPERIMENT NO. 14.

B. diphtheriæ.

Culture, "Park, No. 8;" twenty-four hours growth in sterilized milk. Reaction of milk, 0.108 per cent acid.

The milk at 31.5° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in glycerin agar.
At start	31.5	Growth	1,800
1½ minutes after immersion	55	No growth	None.
2 minutes after immersion	57	do	None.
2½ minutes after immersion	59	do	None.
3 minutes after immersion	60	do	None.
4 minutes after immersion	1 minute at 60	do	None.

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[a] Sternberg: Manual of bacteriology, 1892, p. 148; also, American Public Health Association, committee on disinfectants, p. 152.

EXPERIMENT NO. 15.

B. diphtheriæ.

Culture, "Park"; forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 28° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	28	Growth	3,150
1⅓ minutes after immersion	45	do	1,890
1½ minutes after immersion	48	do.[a]	[b]19
1¾ minutes after immersion	50	do.[a]	26
2 minutes after immersion	51	No growth	None.
2¼ minutes after immersion	52	do	None.
2⅓ minutes after immersion	53	do	None.
2¾ minutes after immersion	54	do	None.
3½ minutes after immersion	55	do	None.

[a] Delayed. [b] Contaminated.

EXPERIMENT NO. 16.

B. diphtheriæ.

Culture, "Park, No. 8;" fifty-two hours' growth in sterilized milk.

Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Three test-tube cultures tested by planting a loop over into bouillon.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.		
		Reaction, 0.171 per cent acid.	Reaction, 0.135 per cent acid.	Reaction, 0.135 per cent acid.
At start	28.5	Growth	Growth	Growth.
1⅓ minutes after immersion	50	No growth		
2½ minutes after immersion	55	do	No growth	No growth.
2⅔ minutes after immersion	58	do	do	Do.
4⅓ minutes after immersion	60	do	do	Do.
5⅓ minutes after immersion	(a)	do	do	Do.

[a] One minute at 60°.

EXPERIMENT NO. 17.

B. diphtheriæ.

Culture, "Park, No. 8;" forty-eight hours' growth in sterilized milk. Reaction of milk, 0.162 per cent acid.

The milk at 25° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	25	Growth.
1½ minutes after immersion	45	Do.
2 minutes after immersion	50	No growth.
2¼ minutes after immersion	51	Do.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53	Do.
3 minutes after immersion	54	Do.
3¾ minutes after immersion	55	Do.
4¾ minutes after immersion	1 minute at 55	Do.
5¾ minutes after immersion	2 minutes at 55	Do.
6¾ minutes after immersion	3 minutes at 55	Do.

EXPERIMENT NO. 18.

B. diphtheriæ.

Culture, "Park, No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.135 per cent acid.

The milk at 30° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.	Loop of milk culture in in bullion.
At start	30	Growth	Growth.
1⅓ minutes after immersion	45	do	Do.
1½ minutes after immersion	48	do	Growth, delayed.
1¾ minutes after immersion	50	do	Growth, small, delayed.
2 minutes after immersion	51	do	Growth, delayed.
2⅓ minutes after immersion	52	do	Growth, small, delayed.
2¾ minutes after immersion	53	do	No growth.
4 minutes after immersion	54	Growth, delayed.	Do.
5¾ minutes after immersion	55	do	Do.

This experiment demonstrates the different results obtained by carrying over large and small quantities of the test fluid.

EXPERIMENT NO. 19.

B. diphtheriae.

Culture, "Park:" forty-eight hours' growth in sterilized milk. Reaction, 0.09 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
1¾ minutes after immersion	53	Do.
2¼ minutes after immersion	55	Do.
2½ minutes after immersion	56	Do.
2¾ minutes after immersion	58	Growth, small.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	No growth.
6 minutes after immersion	2 minutes at 60	Do.

EXPERIMENT NO. 20.

B. diphtheriae.

Culture, "Park, No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	0.5 c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	No growth.
3 minutes after immersion	58	Do.
3½ minutes after immersion	59	Do.
4¾ minutes after immersion	60	Do.
5¾ minutes after immersion	1 minute at 60	Do.
6¾ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT No. 21.

B. diphtheriae.

Culture, "Park, No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	0.5 c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1¾ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
2¾ minutes after immersion	57	No growth.
3¼ minutes after immersion	58	Do.
4 minutes after immersion	59	Do.
5½ minutes after immersion	60	Do.
6½ minutes after immersion	60.4	Do.
7½ minutes after immersion	60.5	Do.

EXPERIMENT No. 22.

B. diphtheriae.

Culture, "Park, No. 8;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.216 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2½ minutes after immersion	55	No growth.
2¾ minutes after immersion	57	Do.
3¾ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

Conclusion.—The diphtheria bacilli succumb at comparatively low temperatures. Oftentimes they fail to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

VIBRIO CHOLERÆ.

REVIEW OF THE WORK OF OTHERS.

On account of its acid reaction milk is not a suitable medium for cholera.

Van Geuns,[a] 1889, showed that 59° C. for one minute, or 54° for five minutes, was ample to kill Koch's comma bacillus.

Sternberg,[b] 1888, found that a temperature of 52° C. (125.6° F.) was sufficient to kill the *Spirillum choleræ Asiaticæ*. For details of Sternberg's technique see page 48.

Kitasato,[c] 1889, tested the thermal death point of the cholera bacillus in gelatin and found that it required 60° C. for ten minutes to kill this organism with certainty, although sometimes it dies at 50° for fifteen minutes or 55° for ten minutes. Kitasato used eight different cultures and made a number of experiments, the results of which are brought together in the following table:

	I.	III.	VI.	VIII.	IX.	X.	XII.	XIV.
50° C., 5 minutes	+	+	+	+	+	+	+	+
50° C., 10 minutes	+	+	+	+	+	+	+	+
50° C., 15 minutes	+	+	+	+	−	+	−	−
55° C., 5 minutes	+	+	+	+	−	−	−	+
55° C., 10 minutes	+	+	+	+	−	+	−	−
55° C., 15 minutes	−	+	+	−	−	−	−	−
60° C., 5 minutes	−	−	+	−	−	−	−	−
60° C., 10 minutes	−	−	−	−	−	−	−	−
65° C., 5 minutes	−	−	−	−	−	−	−	−

The method used by Kitasato was first to transfer a platinum loop of the culture into a test tube containing gelatin which had previously been melted. This tube was then introduced into a water bath which had been brought up to the temperature required. After the time stated, the gelatin tubes were rolled and incubated at 20° to 22° C. Kitasato found no difference between old and young cultures of cholera so far as their resistance to heat was concerned, and further concluded that no material difference was evident so far as the resistance of cholera cultures are concerned against temperatures of 50° to 60°.

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[a] Van Geuns: Über des Pasteurisiren von Bakterien. Ein Beiträg zur Biologie der Microorganismen. Arch. f. Hyg., vol. 9, 1889, p. 369.

[b] Sternberg: Manual of Bacteriology, 1892, p. 146, and Report of the Committee on Disinfectants of the American Public Health Association, pp. 136, 152.

[c] Kitasato, S.: Die Widerstandsfähigkeit der Cholerabacterien gegen das Eintrocknen und gegen Hitze. Zeit. f. Hyg., vol. 5, 1889, p. 135.

Friedel, Kutscher, and Meinicke,[a] 1904, found that the cholera vibrio was not killed when the temperature reached 58°, but was dead as soon as 60° was reached. It required seven to eight minutes for the milk to reach 60°.

These experiments with the cholera organism were conducted with and without the enrichment methods. The enrichment methods consist of first planting a milk culture in peptone water, incubating twenty-four hours, and then plating into litmus-lactose-agar. With the enrichment method it was found that cholera withstood sixty minutes at 50°, but only twenty-five minutes without the enrichment method. At 53° it withstood forty minutes with and fifteen minutes without the enrichment method.

ORIGINAL EXPERIMENTS.

In my work I used three different cultures with the following results:

EXPERIMENT No. 23.

B. cholera.

Forty-eight hours' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature ° C.	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
"Manila;" reaction, 0.180 per cent acid:			
At start	28	Growth	450,000
1½ minutes after immersion	45	do	3,750
1⅔ minutes after immersion	48	No growth	[b]10
1¾ minutes after immersion	50	do	None.
2 minutes after immersion	51	do	None.
2¼ minutes after immersion	52	do	None.
2¾ minutes after immersion	53	do	None.
3 minutes after immersion	54	do	None.
3¾ minutes after immersion	55	do	None.
"Hamburg;" reaction, 0.229 per cent acid:			
At start	28	Growth	87,500
1½ minutes after immersion	45	do	144
1⅔ minutes after immersion	48	No growth	4
1¾ minutes after immersion	50	do	None.
2 minutes after immersion	51	do	None.
2¼ minutes after immersion	52	do	None.
2¾ minutes after immersion	53	do	None.
3 minutes after immersion	54	do	None.
3¾ minutes after immersion	55	do	None.

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[a] Friedel, Kutscher, and Meinicke: Die Widerstandsfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 13, 1904-05, p. 324.

[b] Contaminated.

EXPERIMENT No. 24.

B. choleræ.

Three days' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
"Manila;" reaction, 0.219 per cent acid:			
At start	28	Growth	[1]
1¼ minutes after immersion	50	Lost	
2 minutes after immersion	55	No growth	
3 minutes after immersion	58	do	None.
5½ minutes after immersion	60	do	None.
"Paris;" reaction, 0.189 per cent acid:			
At start	28	Growth	[1]
1¼ minutes after immersion	50	No growth	
2 minutes after immersion	55	do	
3 minutes after immersion	58	do	None.
5½ minutes after immersion	60	do	None.

[1] Innumerable.

EXPERIMENT No. 25.

B. choleræ.

Culture, "Hamburg;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.216 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	[1]
1⅛ minutes after immersion	45	41
1⅜ minutes after immersion	48	None.
1¾ minutes after immersion	50	None.
2 minutes after immersion	51	None.
2¼ minutes after immersion	52	None.
2½ minutes after immersion	53	None.
3 minutes after immersion	54	None.
4 minutes after immersion	55	None.

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[1] Innumerable.

EXPERIMENT No. 26.

B. choleræ.

Culture, "Manila;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.210 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	(a)
1 minute after immersion	45	6,600
1¼ minutes after immersion	48	628
1¾ minutes after immersion	50	546
2 minutes after immersion	51	0
2¼ minutes after immersion	52	0
2¾ minutes after immersion	53	0
4 minutes after immersion	54	0
5 minutes after immersion	55	0

[a] Innumerable.

EXPERIMENT No. 27.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(a)
1½ minutes after immersion	45	1,164
2 minutes after immersion	50	32
2½ minutes after immersion	52	0
2¾ minutes after immersion	53	0
3¾ minutes after immersion	54	0
5 minutes after immersion	55	0
6 minutes after immersion	1 minute at 55	0
7 minutes after immersion	2 minutes at 55	0
8 minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 28.

B. choleræ.

Culture "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.198 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature °C.	Number colonies per loop in gelatin.
At start	24	[a]
1½ minutes after immersion	47	[b]
2 minutes after immersion	50	245
2¼ minutes after immersion	52	205
3 minutes after immersion	53	22
3¼ minutes after immersion	54	0
4¾ minutes after immersion	55	0
5¾ minutes after immersion	1 minute at 55	0
6¾ minutes after immersion	2 minutes at 55	0
7¾ minutes after immersion	3 minutes at 55	0

[a] Innumerable. [b] Innumerable, but less.

EXPERIMENT No. 29.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature °C.	Number colonies per loop in gelatin.
At start	24	[a]
1½ minutes after immersion	47	1,680
2 minutes after immersion	50	244
2¼ minutes after immersion	52	47
2¾ minutes after immersion	53	0
3¼ minutes after immersion	54	0
4¾ minutes after immersion	55	0
5¾ minutes after immersion	1 minute at 55	0
6¾ minutes after immersion	2 minutes at 55	0
7¾ minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 30.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.181 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(a)
1½ minutes after immersion	45	4,455
2 minutes after immersion	50	1,100
2¼ minutes after immersion	52	90
3 minutes after immersion	53	7
3¾ minutes after immersion	54	0
5¼ minutes after immersion	55	0
6¼ minutes after immersion	1 minute at 55	0
7¼ minutes after immersion	2 minutes at 55	0
8¼ minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 31.

B. choleræ.

Culture, "Hamburg;" two days' growth in sterilized milk. Reaction, 0.189 per cent acid.

Milk immersed at 25.3° C. in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	25.3	Growth.
1½ minutes after immersion	45	Do.
2 minutes after immersion	50	No growth.
2¼ minutes after immersion	51	Do.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53	Do.
3¼ minutes after immersion	54	Do.
4½ minutes after immersion	55	Do.
5½ minutes after immersion	1 minute at 55	Do.
6½ minutes after immersion	2 minutes at 55	Do.
7½ minutes after immersion	3 minutes at 55	Do.

Experiment No. 32.

B. choleræ.

Culture, "Manila:" forty-eight hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

Milk at 26.3° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	26.3	Growth.
1¼ minutes after immersion	45.	Do.
1¾ minutes after immersion	50.	Do.
2¼ minutes after immersion	51	No growth.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53.	Do.
3¼ minutes after immersion	54	Do.
4¼ minutes after immersion	55.	Do.
5¼ minutes after immersion	1 minute at 55	Do.
6¼ minutes after immersion	2 minutes at 55	Do.
7¼ minutes after immersion	3 minutes at 55.	Do.

Experiment No. 33.

B. choleræ.

Culture, "Hamburg:" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.216 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.
At start	31	Growth.
1 minute after immersion	45	Do.
1½ minutes after immersion	48	No growth.
1⅔ minutes after immersion	50	Growth.
1¾ minutes after immersion	51	No growth.
2 minutes after immersion	52	Growth.
2¼ minutes after immersion	53	No growth.
3 minutes after immersion	54	Do.
4⅔ minutes after immersion	55	Do.

Experiment No. 34.

B. choleræ.

Culture, "Manila:" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.210 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.
At start	31	Growth.
1 minute after immersion	45	Do.
1½ minutes after immersion	48	Do.
1⅔ minutes after immersion	50	Do.
1¾ minutes after immersion	51	Do.
2 minutes after immersion	52	Do.
2⅓ minutes after immersion	53	Do.
3 minutes after immersion	54	(Lost.)
4⅔ minutes after immersion	55	No growth.

EXPERIMENT No. 35.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in sterilized milk. Reaction, 0.140 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1⅔ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2¼ minutes after immersion	55	Do.
2¾ minutes after immersion	57	No growth.
3½ minutes after immersion	59	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT No. 36.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in sterilized milk. Reaction, 0.09 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1⅔ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2¼ minutes after immersion	55	No growth.
2¾ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT No. 37.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in sterilized milk. Reaction, 0.189 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	26	Growth.
1½ minutes after immersion	52	Do.
2 minutes after immersion	55	Do.
5 minutes after immersion	60	No growth.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

Experiment No. 38.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in sterilized milk. Reaction, 0.1[illegible] per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature ° C.	1 c. c. milk culture in bouillon.
At start	26.	Growth.
1½ minutes after immersion	52.	Do.
2 minutes after immersion	55.	Do.
5 minutes after immersion	60.	Do.
6 minutes after immersion	1 minute at 60.	No growth.
7 minutes after immersion	2 minutes at 60.	Do.
8 minutes after immersion	3 minutes at 60.	Do.

Experiment No. 39.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.243 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature ° C.	1 c. c. milk culture in bouillon.
At start	21.	Growth.
1¼ minutes after immersion	50.	Do.
1½ minutes after immersion	53.	Do.
1¾ minutes after immersion	55.	Do.
2¾ minutes after immersion	57.	No growth.
3 minutes after immersion	59.	Do.
4½ minutes after immersion	60.	Do.
5½ minutes after immersion	1 minute at 60.	Do.
6½ minutes after immersion	2 minutes at 60.	Do.
7½ minutes after immersion	3 minutes at 60.	Do.

Experiment No. 40.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.234 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature ° C.	1 c. c. milk culture in bouillon.
At start	21	Growth
1½ minutes after immersion	50	Do.
2 minutes after immersion	53.	Do.
2¼ minutes after immersion	55.	No growth
2¾ minutes after immersion	57.	Do
3½ minutes after immersion	59.	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

Conclusion.—The cholera vibrio is similar to the diphtheria bacillus so far as its thermal death point is concerned. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

BACILLUS DYSENTERIÆ.

The only reference to the thermal death point of the *B. dysenteriæ* I have been able to find is the work done under Kolle's direction by Friedel, Kutscher, and Meinicke,[a] who found that the dysentery bacillus withstands a temperature of 60° for eight minutes, but is killed at 65° for two minutes. In these experiments it required five minutes to heat the milk from 15° C. to the temperature noted.

I used eight different cultures of the dysentery bacillus, representing both the Shiga and the Flexner types. My results are given in the following tables:

Experiment No. 41.

B. Dysenteriæ.

Three days' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bullion.	Number of colonies per loop in agar.
"Duval;" reaction, 0.126 per cent acid:			
At start	28	Growth	(b)
1¼ minutes after immersion	55	do	1,800
2½ minutes after immersion	58	do	28
3¾ minutes after immersion	60	No growth	0
4¾ minutes after immersion	(c)	do	0
"Sils;" reaction, 0.162 per cent acid:			
At start	29	Growth	(b)
1⅝ minutes after immersion	55	do	1,110
2¼ minutes after immersion	58	do.[d]	56
3 minutes after immersion	60	No growth	0
4 minutes after immersion	(c)	do	0
"Kruse;" reaction, 0.187 per cent acid:			
At start	28	Growth	(b)
1⅔ minutes after immersion	55	do	126
2⅓ minutes after immersion	58	do.[d]	0
3⅔ minutes after immersion	60	No growth	0
4⅔ minutes after immersion	(c)	do	0
"Mt. Desert;" reaction, 0.198 per cent acid:			
At start	28	Growth	(b)
1¾ minutes after immersion	55	do	57
2½ minutes after immersion	58	No growth	14
4¼ minutes after immersion	60	do	0
5¼ minutes after immersion	(c)	do	0
"Salant;" reaction, 0.189 per cent acid:			
At start	29	Growth	(b)
1½ minutes after immersion	55	do	(b)
2¼ minutes after immersion	58	do	154
3⅔ minutes after immersion	60	No growth	0
4⅔ minutes after immersion	(c)	do	0

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[a] Friedel, Kutscher, and Meinicke: Die Widerstandsfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 3, 1904–5, p. 34.

[b] Innumerable.

[c] One minute at 60°.

[d] Delayed.

EXPERIMENT NO. 42.

B. dysenteriæ.

Culture, "Salent;" forty-eight hours' growth in sterilized milk. Reaction, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1¾ minutes after immersion	50	Do.
2½ minutes after immersion	55	Do.
3 minutes after immersion	57	Do.
3½ minutes after immersion	58	Do.
4½ minutes after immersion	59	Do.
5¼ minutes after immersion	59	Do.
6¼ minutes after immersion	60	Do.
7¼ minutes after immersion	1 minute at 60	Do.

EXPERIMENT NO. 43.

B. dysenteriæ.

Culture, "Sils;" forty-eight hours' growth in sterilized milk. Reaction, 0.176 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3 minutes after immersion	58	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.

EXPERIMENT No. 44.

B. dysenteriæ.

Culture, "Salant;" forty-eight hours' growth in sterilized milk. Reaction, 0.135 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	½ c. c. milk culture in bouillon.
At start	24	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¾ minutes after immersion	60	Do.
4¾ minutes after immersion	1 minute at 60	Do.
5¾ minutes after immersion	2 minutes at 60	No growth.
6¾ minutes after immersion	3 minutes at 60	Do.
7¾ minutes after immersion	4 minutes at 60	Do.
8¾ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT No. 45.

B. dysenteriæ.

Culture, "Salant;" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
2 minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	No growth.
13 minutes after immersion	8 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.
20 minutes after immersion	15 minutes at 60	Do.

EXPERIMENT No. 16.

B. dysenteriæ.

Culture "Duval:" forty-eight hours' growth in sterilized milk. Reaction, 0.108 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature C.	½ c. c. milk culture in bouillon.
At start	26.	Growth.
1½ minutes after immersion	50.	Do.
2½ minutes after immersion	55.	Do.
3½ minutes after immersion	58.	Do.
4½ minutes after immersion	60.	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.
7½ minutes after immersion	3 minutes at 60	No growth.
9½ minutes after immersion	5 minutes at 60	Do.
14½ minutes after immersion	10 minutes at 60	Do.

EXPERIMENT No. 17.

B. dysenteriæ.

Culture. "Sils:" forty-eight hours' growth in sterilized milk. Reaction, 0.165 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature °C.	½ c. c. milk culture in bouillon.
At start	26	Growth
2 minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	No growth.
7 minutes after immersion	2 minutes at 60	Do
10 minutes after immersion	5 minutes at 60	Do
13 minutes after immersion	8 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.
20 minutes after immersion	15 minutes at 60	Do.

EXPERIMENT No. 48

B. dysenteriæ.

Culture, "Kruse;" forty-eight hours' growth in sterilized milk. Reaction, 0.144 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. °C.	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1½ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
3¾ minutes after immersion	60	Do.
4¾ minutes after immersion	60.5	No growth.
5¾ minutes after immersion	61	Do.
6¾ minutes after immersion	61.5	Do.
7¾ minutes after immersion	61.5	Do.
8¾ minutes after immersion	61	Do.

EXPERIMENT No. 49.

B. dysenteriæ.

Culture, "Witaus;" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature °C.	½ c. c. milk culture in bouillon.
At start	25	Growth.
1½ minutes after immersion	50	Do.
2 minutes aftes immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	No growth.

EXPERIMENT No. 50.

B. dysenteriæ.

Culture, "Wilson;" forty-eight hours' growth in sterilized milk. Reaction, 0.144 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature °C.	½ c. c. milk culture in bouillon.
At start	25.	Growth.
1¼ minutes after immersion	50.	Do.
2 minutes after immersion	55.	Do.
2¾ minutes after immersion	58.	Do.
5 minutes after immersion	60.	Do.
6 minutes after immersion	1 minute at 60.	Do.
7 minutes after immersion	2 minutes at 60.	Do.
8 minutes after immersion	3 minutes at 60.	Do.
10 minutes after immersion	5 minutes at 60.	Do.
15 minutes after immersion	10 minutes at 60.	No growth.

EXPERIMENT No. 51.

B. dysenteriæ.

Culture, "Mt. Desert;" forty-eight hours' growth in sterilized milk. Reaction, 0.252 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature °C.	½ c. c. milk culture in bouillon.
At start	26.	Growth.
1¼ minutes after immersion	50.	Do.
2 minutes after immersion	55.	Do.
2½ minutes after immersion	58.	Do.
5 minutes after immersion	60.	Do.
6 minutes after immersion	1 minute at 60.	Do.
7 minutes after immersion	2 minutes at 60.	Do.
8 minutes after immersion	3 minutes at 60.	Do.
10 minutes after immersion	5 minutes at 60.	No growth.
15 minutes after immersion	10 minutes at 60.	Do.

EXPERIMENT No. 52.

B. dysenteriæ.

Culture, "Harris;" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2¾ minutes after immersion	58	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	Do.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	No growth.
14¼ minutes after immersion	10 minutes at 60	Do.

EXPERIMENT No. 53.

B. dysenteriæ.

Culture, "Sils;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.288 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3¼ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	No growth.
15 minutes after immersion	10 minutes at 60	Do.

EXPERIMENT No. 54.

B. dysenteriæ.

Culture, "Shiga:" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.27 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3¼ minutes after immersion	59	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	Do.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	Do.
14¼ minutes after immersion	10 minutes at 60	No growth.

EXPERIMENT No. 55.

B. dysenteriæ.

Culture, "Duval:" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.306 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	No growth.
10 minutes after immersion	5 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.

Conclusion—The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at 60° for five minutes. All are killed at 60° C. for ten minutes. However, the great majority of these micro-organisms are killed by the time the milk reaches 60° C.

MICROCOCCUS MELITENSIS.

Maj. W. H. Horrocks and Capt. J. C. Kennedy, of the British Army Medical Corps, in their article upon goats as a means of propagating Mediterranean fever,[a] made several experiments to determine whether it is possible to destroy the *M. melitensis* by pasteurization of the infected milk, and concluded that heating the milk to 68° C. for ten minutes destroyed the *M. melitensis* present in infected goats' milk. No record, however, is made of experiments with lower temperatures.

Experiment 1.—Milk was drawn from goats 1, 2, 3, 5, and 6 and thoroughly mixed. This mixed milk was selected for experiment as it contained thick, ropy masses, and it was thought that these might act as protecting envelopes to the specific micrococci, so that if such a milk were sterilized by pasteurization it might safely be concluded that any ordinary normal-looking milk would be equally sterilized by the same operation.

One loopful of the mixed milk was stroked on a series of surface plates to act as a control. The mixed milk was then heated on a water bath to 68° C., and this temperature was maintained for ten minutes. The milk was then rapidly cooled and 10 c. c. of the sterilized milk were spread over 20 litmus-nutrose-agar plates.

Results: After four days incubation at 37° C. the control plates were found densely crowded with colonies of the *M. melitensis;* the plates made from the pasteurized milk remained free from any signs of the specific *Micrococcus melitensis*, even though the incubation was continued for fourteen days.

Experiment 2. This served as a control of experiment 1, the same procedure being followed. The *Micrococcus melitensis* appeared in the control plates, but the plates made with the pasteurized milk again remained perfectly sterile.

In my experiments I used two strains of the *M. melitensis.* The results follow:

EXPERIMENT NO. 56.

M. melitensis.

Culture, seven days' growth in bouillon, mixed with milk that had been inoculated the previous day with same culture. Reaction of milk, 0.081 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	Innumerable.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	48	Do.
1¾ minutes after immersion	50	Do.
2 minutes after immersion	51	Do.
2¼ minutes after immersion	52	Do.
2½ minutes after immersion	53	Do.
3 minutes after immersion	54	Do.
4 minutes after immersion	55	Do.

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[a] Horrocks, W. H., and Kennedy, J. C.: VI. Goats as a means of propagation of Mediterranean fever. Reports of the commission * * * for the investigation of Mediterranean fever * * * Part 4. London, Harrison & Sons, Feb., 1906. 8° Paper. 187 p.

EXPERIMENT NO. 57.

M. melitensis.

Culture, seven days' growth in bouillon, mixed with milk that had been inoculated the previous day with same culture. Reaction of milk, 0.081 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c.c of milk culture into bouillon.	Loop of milk culture in bouillon.
At start	31	Growth.	Growth.
1 minute after immersion	45	do	Do.
1½ minutes after immersion	48	do	Do.
1⅔ minutes after immersion	50	do	Do.
2 minutes after immersion	51	do	Do.
2¼ minutes after immersion	52	do	Do.
2½ minutes after immersion	53	do	Do.
3 minutes after immersion	54	do	Do.
4¼ minutes after immersion	55	do	Do.

EXPERIMENT NO. 58.

M. melitensis.

Five days' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Number of colonies per loop in agar.
"Stock" culture; reaction, 0.108 per cent acid:		
At start	30	Innumerable.
½ minute after immersion.	45	Do.
1 minute after immersion.	50	Do.
1¼ minutes after immersion.	55	Do.
1¾ minutes after immersion.	58	53.
2 minutes after immersion.	60	0
3 minutes after immersion.	1 minute at 60	0
"U. S. N." culture; reaction, 0.072 per cent acid:		
At start	33	Innumerable.
½ minute after immersion.	45	Do.
1 minute after immersion.	50	Do.
1⅓ minutes after immersion.	55	Do.
2 minutes after immersion.	58	Do.
2¾ minutes after immersion.	60	0
3¼ minutes after immersion.	1 minute at 60	0

NOTE.—Agar plates incubated five days at 37° C.

Conclusion.—So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. The *M. melitensis* is not destroyed at 55° for a short time; the great majority die at 58°; at 60° all are killed.

GENERAL SUMMARY AND CONCLUSIONS.

It is difficult to determine precisely at what moment or at what temperature a micro-organism dies. The fact that a micro-organism will not grow upon artificial media is not always a sure sign that it is dead; but with the exception of the tubercle bacillus it is safe to assume that ordinarily bacteria that fail to vegetate upon suitable media under favorable conditions have at least lost their virulence and power to infect, especially when ingested by the mouth.

We know, however, that the vegetability of micro-organisms *in vitro* does not always correspond to their ability to grow in the animal host. This is especially true of the tubercle bacillus. On account of the reluctance with which this bacillus grows upon culture media, it is necessary to resort to animal inoculations in order to determine its thermal death point. It is proper to assume that the tubercle bacilli in milk which are so attenuated as to be unable to cause tuberculosis when injected into the peritoneal cavity of a young guinea pig would be harmless when ingested by man.

In these experiments upon the guinea pig it is important to differentiate the lesions produced by dead tubercle bacilli, which closely simulate those caused by the live tubercle bacilli. In doubtful cases it is necessary to inoculate the products of the lesions into another animal to determine the presence or absence of living micro-organisms.

The cultural experiments upon thermal death points are surrounded by many sources of error and numerous pitfalls, all of which must be avoided.

My experiments were designed to imitate the conditions of practical pasteurization. The micro-organisms were heated in open test tubes and the temperature and other factors accurately controlled. Scum formation was disregarded, as it was my intention to reach results that might be applied with confidence in practical pasteurization on a large scale against natural difficulties.

My results of nine series of tests upon guinea pigs with five cultures plainly show that in milk the tubercle bacillus loses its virulence and infective power when heated at 60° for twenty minutes; in other words, it may be considered dead. When heated to 65° C. a much shorter time is necessary. These results correspond in the main with those previously recorded by Yersin, Bonhoff, Schroeder, Th. Smith, Russell and Hastings, and Hesse.

It should be remembered that the milk in these tests was very heavily infected with virulent cultures, indicated by the prompt death of the control animals. Milk practically never contains such an enormous amount of infection under natural conditions. It is justifiable, therefore, to assume that if 60° C. for twenty minutes is sufficient to destroy the infectiousness of such milk when injected into the peritoneal cavity of a guinea pig, any ordinary market milk after such treatment would be safe for human use by the mouth so far as tubercle bacilli are concerned.

The lesions produced by a large mass of dead tubercle bacilli may be distinguished by their extent rather than by their character. In doubtful cases secondary inoculation is the only trustworthy method of determining whether the bacilli are alive or dead. The tuberculin test does not differentiate between the live and dead tubercles. Three guinea pigs out of eight having lesions produced by dead tubercle bacilli (killed at 100° C.) died as the result of the subcutaneous inoculation of 2 c. c. tuberculin (O. T.).

The evidence is plain that milk heated to 60° C. and maintained at that temperature for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C., and few survive to 60° C.

The diphtheria bacillus succumbs at comparatively low temperatures. Oftentimes it fails to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

The cholera vibrio is similar to the diphtheria bacillus so far as its thermal death point is concerned. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at 60° C. for five minutes. All are killed at 60° C. for ten minutes. However, the great majority of these micro-organisms are killed by the time the milk reaches 60° C.

So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. The *M. melitensis* is not destroyed at 55° C. for a short time; the great majority of these organisms die at 58°, and at 60° all are killed.

Milk heated to 60° C. and maintained at that temperature for twenty minutes may, therefore, be considered safe so far as conveying infection with the micro-organisms tested is concerned.

NOTICE TO LIBRARIANS AND BIBLIOGRAPHERS CONCERNING THE SERIAL PUBLICATIONS OF THIS LABORATORY.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued.

No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogienic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxin. II. Maternal transmission of immunity to diphtheria toxin and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldeyhde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous Zoological Papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

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